Annals of Agri-Bio Research **28** (1): 39-46, 2023

Impact Score : 0.28 (Scopus)

Antibacterial Effect of Arabic Coffee Extract against Experimentally Infected with *Escherichia coli* in Mice

ASEEL J. MOHAMMAD*, MAY T. FLAYYIH[1] AND INAM B. FALIH[2]

General Directorate of Education in Baghdad, Al-karkh Firsest, Iraq
**(e-mail: la_aaaa@yahoo.com; Mobile: 00964 79016 52623)*

(Received: May 15, 2022; Accepted: June 17, 2022)

ABSTRACT

A phenolic compound in coffee has antimicrobial activities and anti-inflammatory effects. This study investigated firstly the antibacterial effect of coffee phenolic extract through the agar-well diffusion method, and secondly, the role of coffee in the mice hepatic and small bowel (ileum section) post *Escherichia coli* infection with apoptotic cell detection. The agar well diffusion method was used for the concentrations of 50, 100 and 150 mg/ml, respectively. Results showed that the minimum inhibitory concentration of coffee alcohol extract was 150 mg/ml with an inhibition zone of 19 mm. The results of histopathology showed fewer pathological changes in the hepatic and small bowel (ileum section) tissues in a treated grouping. In contrast, the results of apoptosis showed lower apoptotic activity in treated groups in the hepatic and small bowel tissues.

Key words: Arabic coffee, *Escherichia coli*, histopathology, apoptosis, mice

INTRODUCTION

Several researchers have successfully used various plants and their products against many pathogens as a medicinal value since ancient times. Plant product compounds are shown to have antimicrobial activity. Arabic coffee contains caffeine, a polyphenolic compound, chlorogenic acids, diterpenes, and cafestol (Poole *et al.*, 2017), as well as vitamin B3 and magnesium (Mg; Messina *et al.*, 2015). Using a polyphenolic coffee compound is associated with reducing the risk of developing several liver conditions (Setiawan *et al.*, 2015). The coffee composite has many mechanisms that affect in various microbial actions due to the anti-inflammatory, antioxidant, antifibrotic, and anticancer effects (Jeszka-Skowron, 2015). *Escherichia coli* bacteria frequently cause infections in the gastrointestinal. It is a source of diarrhea infection financially about 30% in number in the globe (Ravichandran and Kareemulla, 2018). *E. coli* is regularly resistant to more antibiotics (multi-drug resistant) because the foreword of new antibiotics will not eradicate this difficulty of infections caused by multidrug-resistance bacteria, which is a big health danger, especially when these infections acquire nosocomially (Ridha, 2019). The high prevalence of antibiotic resistance for the *E. coli* isolates was also indicated by Cuba *et al.* (2014). Our study spotlights the effects of coffee on histopathology and apoptosis throughout the hepatic and small bowel (ileum) section in infected mice with *E. coli*.

MATERIALS AND METHODS

Radaalwan coffee was obtained from a local retail market in Baghdad city. Three serial dilutions of 50, 100 and 150 mg/ml of coffee were geared up by suspending 0.5, 1 and 1.5 g, respectively, in 10 ml of 95% ethanol. Every attentiveness was varied drinkable throughout Whatman No.1, and kept in a sterile test tube at the 4°C until used. *E. coli* isolate was found in the patient's distress as of diarrheal infection in Baghdad city during the period from January 2018 to November 2018. The biochemical tests and API 20E system kit were used to diagnose the isolates.

The antibacterial activity of the Arabic coffee extract was tested on the selected organism by Agar well diffusion method. Mueller Hinton agar plates were cultured with 0.1 ml of a 24 h broth culture of bacteria at tuned to 10^7 CFU/ml (0.5 McFarland), then four wells were made and filled with fixed volumes (0.5 ml) of each

[1]Department of Biology, College of Science, University of Baghdad, Iraq.
[2]Department of Pathology, College of Veterinary Medicine, University of Baghdad, Iraq.

concentration 50, 100 and 150 mg/ml. A control well was made in the center full of 95% ethanol. After that, the plates were incubated overnight at 37°C, and the diameter of the inhibition zones was calculated in millimeters. The minimum inhibitory concentration was defined as the lowest concentration of extract that gave limited bacterial growth.

Mice were arbitrarily alienated into four equal groupings as follows:

First group: Six mice were administered 0.2 ml of distilled water orally considering as a control group.

Second group: Six mice were administered with coffee for 21 days (150 mg/ kg B.W orally).

Third infected group: Six mice were injected IP by 0.2 ml/mouse, which had 1.5 x 107 cfu/ ml of *E. coli* and were absent with no treatment.

Fourth infected+treated group: Six mice were treated with coffee [0.2 ml of the coffee extract (150 mg/kg B.W) was orally given during the experiment] and, after seven days injected intraperitoneally (IP) with *E. coli*.

RESULTS AND DISCUSSION

The *E. coli* looking as rod shape colonies (Enterobacteriaceae family) of gamma-proteobacteria was usually motile with peritrichous flagella (Ray and Bhunia, 2014). It produced zones of beta-hemolysis on blood agar and the rosy colonies lactose fermentation on MacConkey agar. It had capacity to produce metallic sheen colonies when cultured on Eosin Methylene Blue agar (Ray and Bhunia, 2014). The API 20E system confirmed that the isolates belonged to *E. coli*. The inhibition zones of the coffee extract against *E. coli* resulted in (11, 15 and 19) mm, due to the three concentrations of alcoholic extract of 50, 100 and 150 mg/ml, respectively (Table 1). The agar well diffusion method showed the supremacy of the attentiveness at 150 mg/ml. This was due to the solubility of a high quantity of active elements, which inhibited bacterial growth. These observations were in accord with those who found that antimicrobial peptides were active against period on to pathogenic bacteria through various mechanisms of action on bacterial cells as the ability to bind to bacterial cell membranes.

Table 1. The inhibition zones of diverse consideration of coffee extract against *E. coli* development

Concentration (mg/ ml) of coffee	*E. coli* inhibition zone in mm (Mean±SE)
50	11±0.54C
100	15±0.47B
150	19±0.47A
95% ethanol	0.00±0.00D

Different capital letters mean significant (P<0.05) results between different concentrations.

The Arabica coffee extract constituents included melanoidins and trigonelline caffeic acid having antibacterial activity. Coffee extract acted as an anti-inflammatory, antibacterial, anti-angiogenic, and anti-oxidative due to the biologically active compounds of coffee extracts which were found as antimicrobial agents (Tsedale *et al.*, 2020). These observations may be attributed to coffee melanoid in shaving the ability to distress at the minimum inhibitory concentration leading to the discharge of intracellular molecules due to the support of the cell membrane of *E. coli*. This suggestion was in conformity that the coffee compound acting as a bactericidal effect associated with their chelating properties on iron due to metal-chelating possessions, as well as who found that the 4 mg/ml of caffeine extract that inhibited the growth of *E. coli*.

In addition, the coffee compounds acted as a bactericidal effect against *E. coli*. These effects may also show a difference depending on the bacterial species; these ideas accord Khan *et al.* (2021), who found that coffee extracts reduced the viable bacterial count for many microbial pathogens. The action of coffee and tea against *Salmonella typhimurium* and *Shigella flexneri* showed that the aqueous extract of coffee and tea decreased the viable bacterial count of *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella typhi, Shigella flexneri, Staphylococcus aureus* and *Staphylococcus epidermidis*. Lu´is *et al.* (2014) indicated that coffee caffeic acid acted as an antibacterial effect that led to cellular damage of *S. aureus*cells.

Infections associated with *E. coli* are often complicated by the significant ability of this pathogen to become resistant to a range of classes of antibiotics throughout the world. The *E. coli* is known for its multidrug resistance due to the outer membrane of the bacterium. The sensitive alertness of the subject can control the appearance and the distribution of resistance by transmitting *E.*

coli from one intestinal tract to another through contaminated food and contact with infected animals or people. Gharajalar and Shahbazi (2020) found that x drug-resistant strains of *E. coli* were being associated with the meanness of antibiotics in food animals position that the over use of these antibiotics was promoting the growth of resistant bacteria with resistance genes that can be transferred to humans.

The histopathological observation in hepatic tissue of G3 (chiliage with *E. coli*) illustrated severe pathological lesions in examined liver sections of the infected group as wide bleeding necrotic areas overflowing with red blood cells and the remnant of necrotic tissue adherence and penetrating (Fig. 1B). In addition, the presence of pyogranuloma accompanied with necrotic foci involving nuclear pyknosis of affected hepatocyte together with the number of apoptotic hepatocytes (Fig. 1C and D). Another section showed a large granulomatous lesion composed mainly of macrophages and lymphocytes with massive hydropic swelling of hepatic cells that was noticed in another section (Fig. 1E).

The main lesion in the small bowel (ileum section) of G3 revealed provocative cell infiltration and weakening of villi with focal mucosal sloughing together with hyperplasia of lymphoid tissue together with numerous apoptotic enterocytes within sloughed mucosai villi (Fig. 3A and B) as contrast with normal section (Fig. 2).

The pathological section revealed a few changes in hepatic tissue in (G4), as multiple MNCs infiltrations associated with vacuolation of adjacent hepatocytes (Fig. 4A). In addition to the proliferation of kupffer cells with

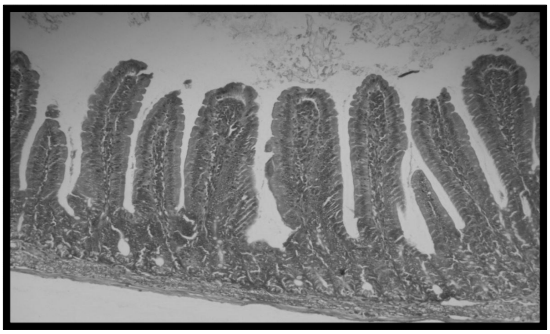


Fig. 2. The histopathological section in the small bowel (ileum section) of (G1) showing usual structural limits (H & E stain 10X).

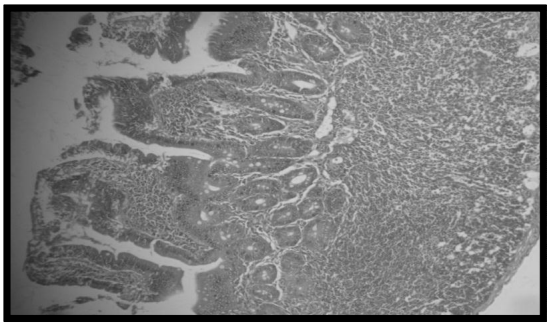
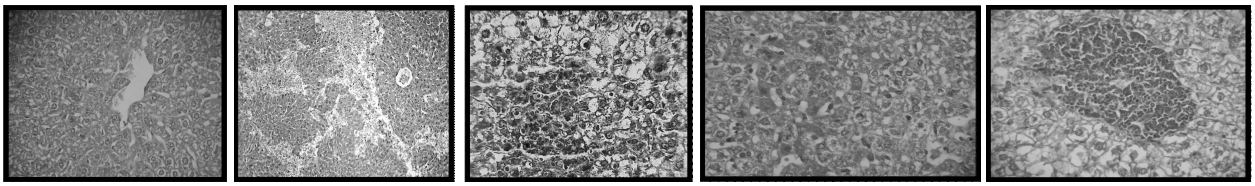


Fig. 3A. Histopathological section in the small bowel (ileum section) of (G3) showing moderate mononuclear cells infiltration in LP with focal mucosal sloughing of some villi: massive enlargement of payers patches (H & E stain X10).

evidence of megakaryocyte with binucleated hepatocytes (Fig. 4B), moreover, perivascular and periductal MNCs aggregation with portal vein distention by edematous (Fig. 4C), while group (G2) showed no obvious lesion (Fig. 5) E stain 40X) 40.

The histopathological section in the small bowel (ileum section) G4 showed mild mononuclear cellular infiltration with well-



A **B** **C** **D** **E**

Fig. 1. Histopathological section in hepatic tissue: A: In control mouse (G1) showing normal structural limits (H & E stain X 10); B: Histopathological section in liver (G3) showing wide hemorrhagic necrotic areas filled with blood cell and cellular debris (H & E stain X 10); C: Histopathological section in liver (G3) showing pyogranulomatous lesion composed of MNCs and neutrophils accompanied with nuclear pyknosis of adjucent hepatocytes (H & E stain X 10); D: Histopathological section in liver (G3) showing numbers of apoptotic hepatocytes (H & E stain X 40) and E: Histopathological section in the liver of (G3) showing large granuloma with massive hydropic welling of hepatic cell (H & E stain x 40).

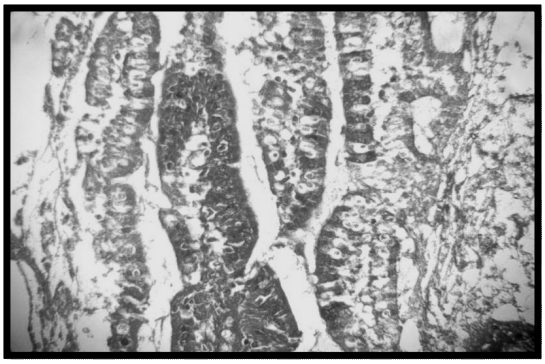


Fig. 3B. Histopathological section in the small bowel (ileum section) of (G3) showing numerous apoptotic enterocytes within sloughed mucosai villi (H & E stain X40).

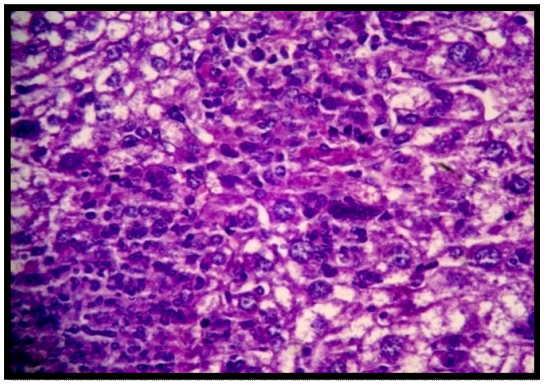


Fig. 4A. Histopathological section in hepatical tissue (G4) showing multiple MNCs infiltrations accompanied by mild vacuolation of some hepatic cells (H & E stain).

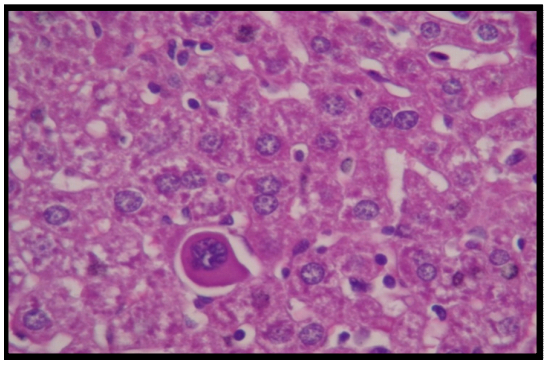


Fig. 4B. Histopathological section in hepatical tissue (G4) showing proliferation of kupffer cells with evidence of megakaryocyte and binucleated hepatocytes (H & E stain X 40).

developed intestinal villi (Fig. 6), while G2 showed no evident action (Figs. 7 and 8).

The study indicated the severe pathological modification in hepatical tissue of infected mice with *E. coli* due to innate activation of

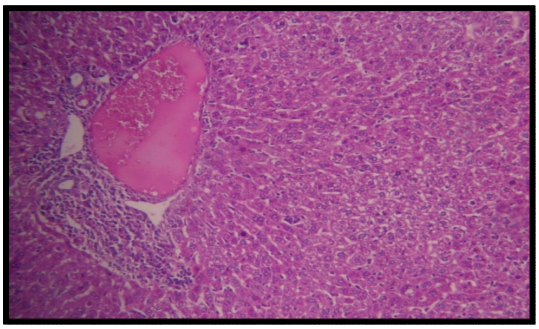


Fig. 4C. Histopathological section in hepatical tissue (G4) showing perivascular and periductal MNCs aggregation with portal vein distention by edematous substance (H & E stain X 10).

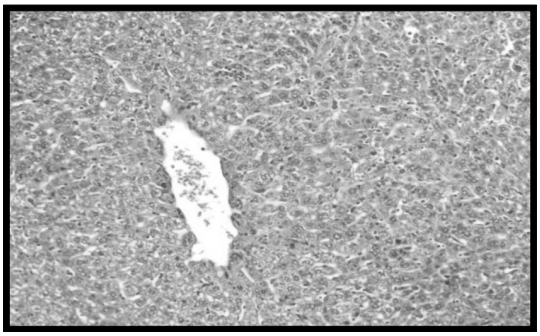


Fig. 5. The histopathological section in hepatical tissue (G2) showing no clear pathologic changes, with the prominence of kupffer cells (H & E stain 10X).

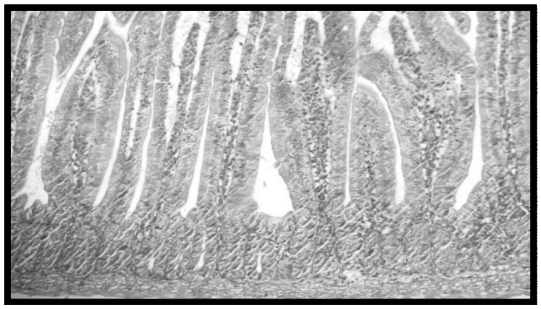


Fig. 6. The histopathological section in the small bowel (ileum section) (G4) showing milled mononuclear cellular infiltration with well-developed villi (H & E stain (40 &10X).

macrophages by microbial products such as LPS that represented potential endotoxin with evidence of enterotoxin effects. The vital accumulation of endotoxinin liver organs after hemorrhagic shock as the porch circulation was the prominent route for endotoxin in the intestine to enter the body after hemorrhagic shock. This idea indicated that *E. coli* was considered a strong indigenous pathogen that

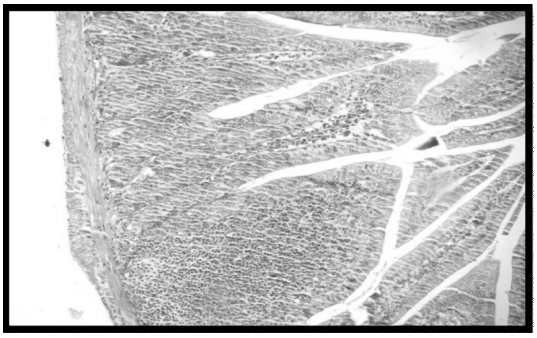


Fig. 7. The section in the small bowel (ileum section) of the mouse (G4) at 21 days post-infected with *E. coli* showing marked hyperplasia of MALT (H & E stain 10X).

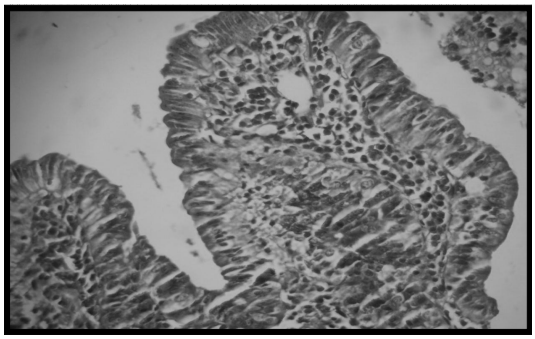


Fig. 8. The section in the ileum of the mouse (G2) at 21 days post-administration coffee showing moderate distension of LP with MNCs infiltration (H & E stain 40X).

caused localized infection of enterocytes that format aching and effacing scratch on the surface of both layers of intestinal layer, matching to the destruction of enterocyte microvilli that facilitated bacterial adherence and invasion of host tissue.

The pathological lesion in the intestines illustrated that the bacteria invaded enterocytes to the proprial tissue to the site of infection, guiding tissue injury and destroying microvilli. These results were consistent with those of Laganenka *et al.* (2020). They reported that the attachment pattern results in severe lesions on the epithelial layer that destroyed microvilli, lack of the absorptive villi, mal absorption of nutrients and diarrhea.

Moderated pathological lesions were evidenced in examined organs of animal-fed diet supplement with coffee post-infection as compared with those of infected group (G3). Ciaramelli *et al.* (2019) found that the coffee compound had a potential of helpful food due to the biochemical possessions. The dietary supplementation with coffee extract improved immune response indicated that coffee compounds acted as an antibacterial effect against numerous bacterial cells. Carmen *et al.* (2015) explained that coffee compound might damage crucial cellular membrane components and protect against cell mutagens that act as a mutagenic antimicrobial activity. The presence of macrophage cells in the treated group with coffee extract referred to the roles of these phagocytic cells in killing the pathogenic bacteria, and these manifestations came in accord with that stated who created that the mononuclear cell possessed an essential role in recognition of pathogenic microorganism.

The results showed higher ratios of apoptotic cells in hepatic and small bowel (ileum section) tissue in the infected grouping (G3; Figs. 9 and 10). In contrast, there was no significant differences in apoptotic cells recorded in hepatic and small bowel (ileum section) tissue in treated grouping (G2; Figs. 11, 12, 13 and 14), and lower apoptotic cell ratios were recorded in hepatic and small bowel (ileum section) tissue of infected and treated grouping (G4; Fig. 15 and 16).

The results demonstrated more apoptotic cells in (G3). These results might be due to *E. coli* having the ability to attack the epithelial cells due to bacterial toxins being able to obliterate

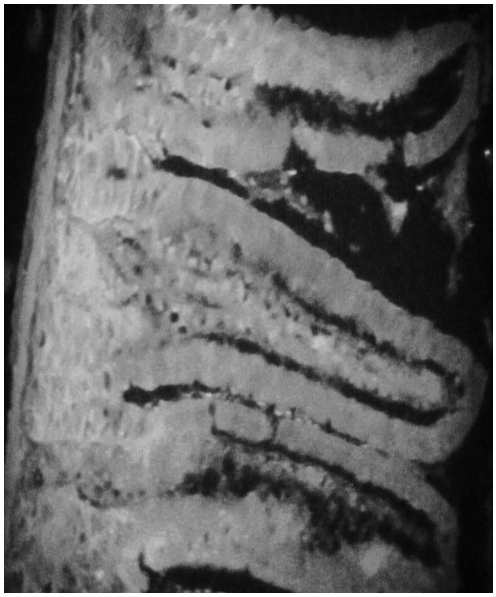


Fig. 9. Part of the small bowel (ileum section) from G1 illustrating number of apoptotic cell (H & E stain 40X).

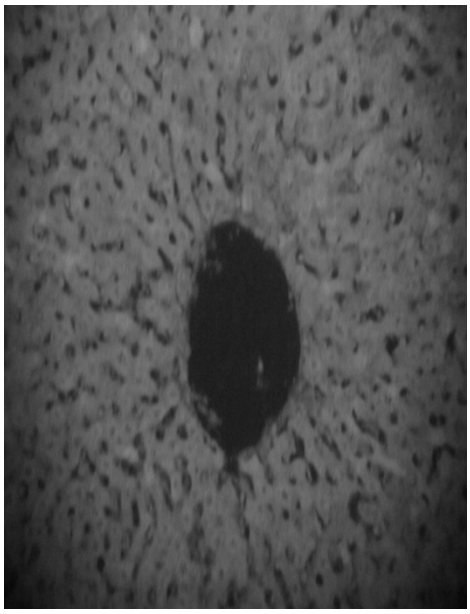


Fig. 10. Part of the hepatica tissue from G1 illustrating no apoptotic cell (H & E stain 40X).

Fig. 11. Part of the hepatica section from G3 illustrating the apoptotic cell becoming visible (H & E stain 40X).

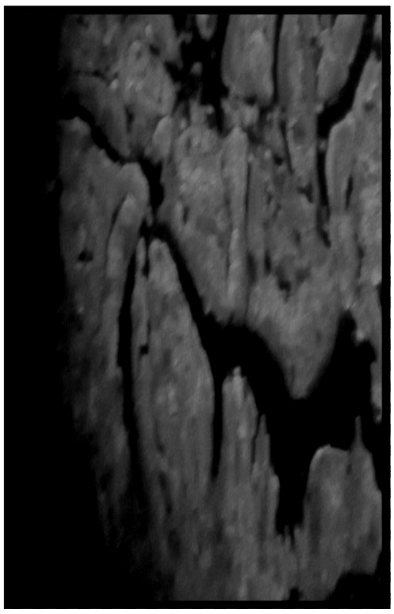


Fig. 12. Part of the small bowel (ileum section) from G3 illustrating the apoptotic cell becoming visible (H & E stain 40X).

Fig. 13. Part of the hepatica section from G2 illustrating that little apoptotic cell becomes visible (H & E stain 40X).

intestinal epithelial cells that go in the circulation cell, which can damage the heart and the liver. The results of apoptosis were in agreement with those who found the main mechanisms responsible for intestinal mucosal injury guide to decreased cellular hyperplasia and increased shrinkage of cells. In addition, enteropathogenic *E. coli* (EPEC) is attached intimately to epithelial cells in the intestine and damages the intestinal lining, that guide to starting on multifaceted sign cascade that finally leads to diarrhea by many devices. These suggestions were in agreement with those who found the EPEC attached to host

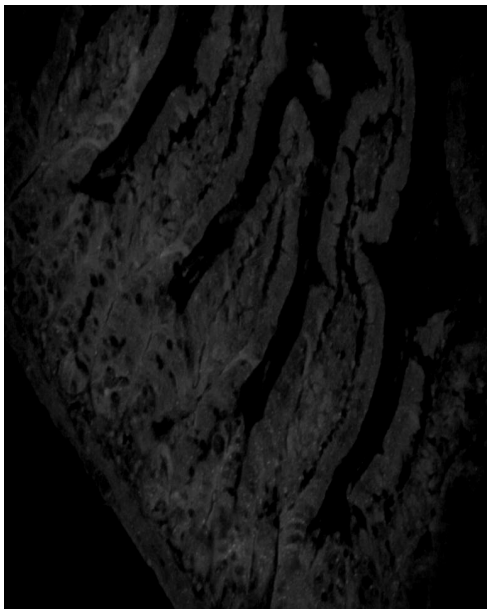


Fig. 14. Part of the small bowel (ileum section) from G2 illustrating that a little apoptotic cell becomes visible (H & E stain 40X).

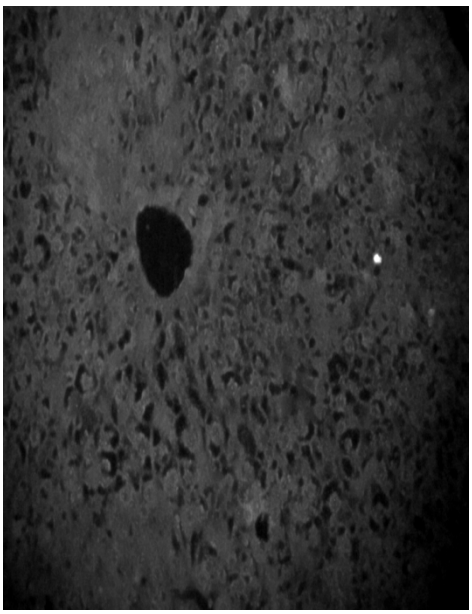


Fig. 15. Part of the hepatica section from G4 illustrating the small number of apoptotic cells becoming visible (H & E stain 40X).

cells, disturbs microvilli, causes act in rearrangements, and infantile diarrhea due to molecular mechanism through the type three secretion systems.

The results also demonstrated the role of coffee extract in reducing bacterial infection by decreasing the ratio of apoptotic cells (G4). This

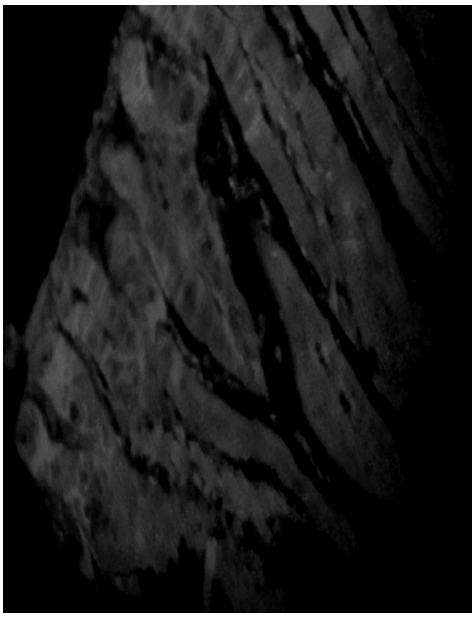


Fig. 16. Part of the small bowel (ileum section) from G4 illustrating the small number of apoptotic cells becoming visible (H & E stain 40X).

could be explained by the fact that coffee compounds act as an antimicrobial activity that leads to lower bacterial migration, damages bacterial cells, and plays the role of defense in wait-free radical-induced cellular damage. Coffee polyphenols and anthocyanin are powerful antioxidants because they can donate a hydrogen atom comparatively free radical. The hydroxyl groups can neutralize the free radicals by forming complexes with them that prevent inflammation reactions. This idea was in concord with those who found that the regular coffee extract has antioxidant activity as evaluated to green and black tea.

Moreover, they suggested that caffeic acid and phenolic acid acted as bactericidal by their radical scavenging activity through interaction plasmas membrane function. These suggestions are in agreement with those who indicated coffee had a major antimicrobial component. In addition, Grosso *et al.* (2017) referred that the coffee complex attributed to helpful effects at the cellular level as to reduce oxidative pressure and injury.

Coffee has a potent antibacterial activity which confirms its use against infection. These observations may be attributed to coffee phenolic compounds these examined in conformity with Brown and Hazen (2015), who found that phenolic compound acted as

antimicrobial due to the relation of heart sickness with the metabolism of microbes in the intestine. Hubert *et al.* (2020) reported that coffee compounds (phenolic phytochemicals) had antioxidant activity, which up-regulated the protective proteins by increasing the expression of genes encoding these proteins, like antioxidant, detoxifying and repair enzymes.

CONCLUSION

The present study supports the use of Arabic coffee compounds as potential antibacterial agents against gastrointestinal infection with *E.coli*, reducing tissue damage, and decreasing free radicals in the hepatic and intestine tissues.

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