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RESEARCH ARTICLE

Correlation Between the Crude Extracellular Secretion by *Shigella dysenteriae* and Destruction of RD and L20B Cell Lines, A Simple Sign as Alternative Treatments for Cancer Tumors through Cytotoxicity

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Abstract

The clinical pathogenic strains of *S. dysenteriae* in this study were initially diagnosed as enteroinvasive *E. coli* due to the high similarity in characterization and pathogenic mechanism. The clinical samples were collected previously from patients of diarrheic stool. Confirmation was done by API 20 NE and API 20 E systems in central public health laboratory in Baghdad. The three clinical strains of *S. dysenteriae* had shown weakness in their ability to produce serine protease autotransporters (Sat), showing small clear zone (about 1-1.4 mm in diameter) around the colonies. No clear hemolysis pattern on blood agar was shown but, the lysis of human Erythrocytes was observed by hemagglutination test in twofold dilution 1/10-1/320 for non-heated bacterial supernatant. When treated with heated supernatant however, agglutination appeared, which indicates that RBCs did not lyse. The biofilm formation was evaluated in this study by Congo red plate Method which had shown weak strength in formation. The clinical strains of *S. dysenteriae* have different antibiogram analyses; it was strong sensitive against ceftriaxone, ciprofloxacin, and co-trimoxazole; while, resistant to Tetracycline, ampicillin and chloramphenicol and finally, Nalidixic acids shown intermediate susceptibility. Both non-heated and heated (at boiling temperature for 60 min) supernatants had cytotoxic effect on shape and vitality of RD and L20B cell lines. Several changes in morphology include: size, shrinkage, segregation, rounding were noted as cytopathic effect. Cell death was shown, which caused either failure of cells in suspension to attach (to a surface to form a monolayer) or detachment of cells from established monolayers within 120 hr. compared with non- treated cells as a control. There is a poor relation between cytotoxicity effect and Sat protease and hemolysin secretion of *S. dysenteriae*. But, other heat-stable toxins may involve in infectivity of *S. dysenteriae* on human cells. Also, the destruction and damage of above mentioned cell lines by extracellular production of clinical pathogens may effect cancer cells when infected with *S. dysenteriae* and become weaker. Cytotoxicity of cancer cells may leave them vulnerable and exposed to active immune cells leading to rapid death. Although the toxins of *S. dysenteriae* are dangerous, they can be employed in treatment of cancer cells or developing anti-tumor drug through destruction of tumor mass.

Keywords: *Shigella dysenteriae*, RD and L20B cell lines, Sat, Cytotoxicity, heated and non-heated supernatant.

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INTRODUCTION

The bacteria and fungi caused different Infectious diseases. World Health Organization (WHO) reports that many hundreds thousand people die daily in the world from infectious diseases (Usha *et al.*, 2010). *Shigella spp*. are pathogenic to humans; it is characterized as fastidious gram-negative, non-spore forming, facultative anaerobic, rod-shaped, non-motile, non-capsulated and non-lactose fermentor (Penatti *et al.*, 2007). Warren and his co-worker reported the most common route of infection by *Shigella* is transmission from person-to-person by the faecal-oral route, as this microbe can survive gastric acidity better than other enteric microorganisms (Warren *et al.*, 2006). However, transmission associated with the consumption of contaminated water, food, food handlers, contaminated swimming pools, overcrowded communities, and flies also has been documented (Ashkenazi, 2004). The bacillary dysentery or called shigellosis that caused by *Shigella* that has the lowest infective dose ranging from 100 to 1000 cell to cause the infection (Chang *et al.*, 2012). The mortality and morbidity due to shigellosis were increased among young children under the age of five, elders and immuno-compromised individuals (Penatti *et al.*, 2007; Singh *et al.*, 2011). Recently, *Shigella* was ranked in the top four bacterial pathogens that cause moderate-to-severe diarrhea (Mani *et al.*, 2016).The genus of *Shigella* was classified into *S. flexneri, S. dysenteriae, S. sonnei and S. boydii* (Niyogi, 2005). The virulent *Shigella* strains cause disease after producing several virulence factors by both chromosomal and plasmid-coded genes and invading the intestinal mucosa; but, rarely penetrates beyond the mucosa (Yang *et al.*,2005).The virulent strains cause cytotoxicity after synthesizing a polypeptide from responsible gene that encoded on large (220 kb) plasmid, the loss or disruption of this plasmid mean the loss of the ability *Shigella* to cause infection. Moreover, *E coli* O157:H7 clinically behave as *Shigella* because it resort this plasmid (Friedrich *et al.*,2002). In many parts of the world, the incidence cases of shigellosis has increased because of emergence Multi-drug resistance *Shigella* spp. Early diagnosis of shigellosis would minimize the risk that caused by *Shigella spp*. (Khalil *et al.*, 1998). The agitation for the bloody diarrhea is caused by lipopolysaccharide (LPS) and Shiga Toxin (an A-B toxin), which acts as a cytotoxin produced by this microbe. There are two groups coded on the chromosome that are considered as non-cross reactive, Stx1 and Stx2. This toxin can also act as a neurotoxin and an enterotoxin (Richardson *et al.*, 1992). LPS antigens in cell wall encoded by chromosomal genes, it plays a remarkable role in reluctance to non-specific lines of host defense which faced throughout tissue infestation. These responsible genes support the invasion, multiplication, and resistance to phagocytosis by tissue macrophages. Some article mentioned that LPS boost the cytotoxicity of Stx on some human cells such as vascular endothelial cells. On the another hand, *S. flexneri* strains also resort responsible gene for other virulence factor, its production of bacteriocin on large plasmid has been described. The production of the bacteriocin that encoded by genes on chromosomes may be related to dysenteric diarrhea (Schuller, 2011). This study's aim was to investigate the correlation of crude extracellular secretion from pathogenic strain of *S. dysenteriae* that may include toxins, protease and hemolysis factors with Cytopathic effect on mammalian cell lines LB20 cells (mouse fibroblast cells) and rhabdomyosarcoma-RD (common tumors cells of soft-tissue sarcomas in skeletal muscle of childhood), which may help find an alternative therapeutic approach to treat tumor cells by cytotoxicity.

MATERIALS AND METHODS

Bacterial isolation and identification

Three *Shigella dysenteriae* isolates were obtained from the Central Public Health Laboratory in Baghdad/Iraq. In order to diagnose the pathogen ,samples cultured directly by streaking on different media include blood agar, MacConkey agar, EMB agar, S.S.agar and Hektoen agar plates after sterilized by autoclave at 121 °C, 15/inch^2 pound for 30 minutes, then incubated in aerobic conditions overnight at 37 °C. The bacterial diagnosis was based on morphology, microscopic characters, and biochemical tests. Confirmation was done by API 20 E and API 20 NE system. The purified bacterial colonies was maintained on LB slant for 3-4 weeks and in LB broth with 20% glycerol at -20°C for long time preservation for subsequent tests.

Hemolytic activity

The hemolytic activity of *S. dysenteriae* isolates were investigated by two methods

1- The classical Blood agar method according to Lin A. and his co-workers with some modification. A single colony of each strain was transported from LB broth onto blood agar containing 5% (v/v) human blood. The hemolysis pattern around the colonies observed after 24 hrs. incubation at 37°C (Lin *et al.*, 2012).

2- Hemagglutination test according to Duguid method (Duguid, 1959). Briefly, RBCs freshly obtained from healthy volunteer were washed 2-3 time with phosphate buffer saline (PBS) and re-suspended at concentration of 2% (vol/vol). bacterial isolates were grown overnight in brain heart infusion broth at 37 °C, washed and suspended in PBS at a concentration of about 1x 10^8 CFU/ml according to turbidity matching 0.5 McFarland standard. Serial twofold dilutions (1/10 - 1/1280) of the bacterial suspension (heated and non-heated) in 25 µl of BPS were made in U-shape microtiter plate. The positive result of hemagglutination was determined after 60 min at 4°C with granulated appearance of RBCs.

Determination of protease production (assay)

The bacterial strains inoculated in LB broth medium and incubated at 37 °C Overnight. The culture broth was subjected to cooling centrifugation for 20 minutes with high speed (10,000 rpm) at 4°C to remove unwanted particles. The supernatant was used as crude enzyme preparation. The bacterial supernatant (20-25µl) was transferred to wells in skim milk agar plates supplemented with 10% skim milk, then incubated at 37 °C for 24-48 hrs. The positive result for protease production based on clear zones around the wells and the diameters of clearance zone was measured with millimeter (Al-Rubai, 2017).

Standardization of inoculum for Antibiotic susceptibility testing

Fresh colonies from plates of nutrient agar at 37°C for 24 h were selected and suspended in Mueller-Hinton broth and then incubated at 37°C for 24 hrs. to a turbidity matching 0.5 McFarland standard (10^8 cfu/ml) for bacteria (McFarland, 1907). The Kirby Bauer method was employed to determine the antibiogram profiles for 7 types of antibiotics against *S. dysenteriae* isolates; the common antibiotics were used provided from Oxoid include (ciprofloxacin 5µg, ampicillin 10 µg, co-trimoxazole 25µg, tetracyclin 30 µg, chloramphenicol 30 µg, ceftriaxone 30µg, and Nalidixic acid 30 µg) , the diameters of inhibition zone for each antibiotics were measured with millimeter and values were indicated as resistant and sensitive categories referring to match chart of national committee for clinical laboratory standard (CLSI, 2018).

Biofilm Formation

It was determine by Congo red plate Method, the medium consisting of Brain heart infusion broth (37 gm/l), sucrose (5 gm/l) and agar number 1 (10 gm/l). Congo red stain was prepared as concentrated solution and autoclaved at 121°C for 15 min. Then it was added to sterilize Brain heart infusion agar supported with sucrose at 55°C. Plates were streaked with *S. dysenteriae* isolates and incubated aerobically at 37°C for 24 - 48 hrs. The positive indicator for biofilm production is black colonies with a dry crystalline consistency (Mathur *et al.*, 2006).

Heat treatment of culture supernatant

S. dysenteriae isolates were inoculated in ten ml of LB broth and allowed to grow for 18 hr. at 37°C in incubator; the bacterial mass was harvested by cooling centrifuge at 10.000 rpm for 20 min at 4°C, the supernatant was sterilized immediately by filtration using 0.2 mm pore size syringe filter unite and then treated with different temperatures 50, 80 and 100°C respectively for 60 min, the filtrate tubes placed immediately in ice bath until cooled and then applied to monolayer of RD and L20B mammalian cell lines (Al-Rubai *et al.*, 2011).

Cytopathic effect

The mammalian L20B and RD were provided from Iraqi National Polio Laboratory in the center public health laboratory of Baghdad, these cells were used to investigate the cytopathogenic effect of bacterial heated and non-heated filtrated of *S. dysenteriae* isolates. The cell lines were maintained according to (Balamurugan *et al.*, 2006) with some modification by (Al-Rubai, 2009). briefly, the cell layers allowed to grown in tissue culture flasks with DMEM supplemented with 10% fetal bovine serum albumin and then different

common use of antibiotics such as gentamycin 40µg/ml , amphotericin B 1 µg/ml , and 0.75 mM L-glutamine were added. The cells washed gently with PBS for 1-4 times and trypsinized the fixed cells by added 0.25% of Trypsin-EDTA solution and then incubated for 6-10 minutes at 36 °C. After trysinization the cells detached and suspended well by shacked gently and then re-suspend in 20-25 ml of DMEM. Finely, distributed to grown in 96- well flat bottom micro titer plate at 37 °C in 5% CO2 incubator for 72 hrs until confluent monolayer formation, the old solution medium was exchanged with 150 µl of new DMEM and then added 50 µl of the heated and non-heated bacterial filtrate to each well and re-incubated in incubator for 120 hrs with same growth condition. The microtiter plate was monitored using the inverted microscope to determine and recorded any changes in morphology of cells may be caused by cell free filtrate and compared with control.

RESULTS AND DISCUSSION

The Three clinical isolates *S. dysenteriae* were collected previously from stool samples of patients suffering from severe diarrhea. The bacterial identification was re-confirmed by API 20 NE and API 20 E systems. The report by Sethabutr and his co-workers mentioned that enteroinvasive *E. coli* (EIEC) is very closely related with *Shigella* species and has a similar pathogenic mechanism. Therefore, it is evident that many enteroinvasive *E. coli* could be called *Shigella* and vice versa, this relatedness makes the differentiation between them difficult and need serotyping for complete identification (Sethabutr *et al.*, 1993; Grimont *et al.*, 2007). For the above reasons**,** only three clinical isolates were diagnosed correctly as *S. dysenteria,* after the initial diagnosis of many stool samples which was EIEC *E.coli* in all the other cases. The reconfirmation and differentiation between these isolates were done in Central Public Health Laboratory in Baghdad. This result indicates that the number of infections with *S. dysenteriae* is low despite the similarity of clinical signs and symptoms with *E. coli*. The asymptomatic carrier of S*. dysenteriae* is believed has an important role in sustaining the organism and in the spread of the disease within susceptible people. Our results were similar to Al-Musawi's research team results that indicated the bacterial contamination with *Shigella* spp. was 3.2% (Al-Musawi *et al.*,2016) on the other hand, during military operation in Iraq, *Shigella spp* was (20%) isolated from US Marines stool samples in14 different battalion (Thornton *et al.*, 2005). The survey research in both Bangladesh and Nigeria showed that the carrier rate of *Shigella spp* in children was 2–3% (Ghosh *et al.*, 2014). However, reports from different Asian countries showed that incidence rates of infection raised by *Shigella* ranging 3 - 13% (Dhodapkar *et al.*, 2008). The diarrheic stool infection that caused by *S. dysenteriae* may attributed to ability of this pathogen to adhere on the intestinal surface and induce diarrhea with pain , but the mortality is low or may cause by secretion such as neurotoxin or enterotoxin from *S. dysenteriae* (Richardson *et al.*, 1992). In spite of the most pathogenic bacteria can produce different proteases and have the potential to breakdown the functional and structural proteins that comprise host tissues as well as to degradation important proteins in host defense. but in our study the ability of *S. dysentria* protease production was weak and small clear zone around the colonies occured with 1-1.4 mm diameter (table 1), the protease of *Shigella* classified into serine protease autotransporters (Sat), it is also, produced by diarrheagenic *E. coli*, it is enumerate among a significant virulence factors in both *E. coli* and *Shigella* (Henderson *et al.*, 1998) which in turn is able to stimulate autoproteolysis of the mature protein from the Sat domain at the bacterial surface (Hendrixson *et al.*, 1997; Stathopoulos *et al.*, 2000) also, the isolates show low hemolysis patterns on blood agar (table 1). But, the lysis of RBCs was observed in moderate intensity by hemagglutination test for non-heated bacterial supernatant in twofold dilution 1/10-1/320, while, agglutination appeared after treating the sample with heated supernatant which indicates that RBCs did not lyse (Table 2). The biofilm formation also was weak. The weakness in results may attributed to different reasons deal with involvement of many of the virulence factors under study or other factors such as growth conditions such as strain variation, origin, incubation temperature or, media constitute.

The agglutination appeared with heated supernatant treatment may attributed to the toxin was consider heat-labile and the lyses action was stopped by high temperature. This result can be

concluded that hemolytic factor consider among virulence factors of *S. dysenteriae* because it lyse red blood cells .Using calcium ions to forming the transmembrane pores in the lipid bilayers of erythrocytes lead to loss of vitality and finely death of cells (Beutin *et al.*,1994). Some authors were reported that hemolysis was occurred by formation of a 25-Å pore within RBCs membrane not by hemolysis enzyme action but to action some proteins of type III secretion system like IpaB and IpaC insertion into RBC membrane. Ipa protein secretion and hemolysis were kinetically coupled processes during RBC-bacterial contact (Blocker *et al.*, 1999).

The results of antibiogram profile were appearing in Table 3. Results of many previous studies for antibiotic susceptibility testing were different; some of antibiotics were combatable and other incompatible with our result. Till now, we do not have a decent explanation for these variation results. *S. dysenteriae* has a variable behavior against different antibiotics. It was sensitive to ceftriaxone, ciprofloxacin and co-trimoxazole respectively; while resistant to tetracycline, chloramphenicol and ampicillin respectively , but Nalidixic acid was appeared moderate activity against *S. dysenteriae* comparatively with control *E. coli* ATCC 25922.

Our results are compatible with the guidelines developed by WHO periodically, it was announced ciprofloxacin is drug of choice for infection by *Shigella* species (WHO, 2005). According to local previous studies for antibiogram analysis by disc diffusion method, the clinical isolates of *Shigella* were isolated from patients whom suffering from diarrhea were resistant to Cefotaxime, Ampicillin, Streptomycin, Nalidixic acid, and Kanamycin (Suhad, 2007) whereas, Asaad K.T. and Afaf A. were noticed resistant to Rifampicin, Ampicillin, and Amoxicillin, but it appeared sensitive to Ciprofloxacin, Nalidixic acid, Neomycin and Cefotaxime (Asaad and Afaf, 2010). The antimicrobial drug profile in Switzerland European country during period 2004–2014 for *S.* dysenteriae were appeared resistance to Amoxicillin/clavulanic acid Cephalothin, Cefotaxime, Ciprofloxacin, Azithromycin, Kanamycin, Gentamicin and Nalidixic acid, but it were shown sensitive to Ampicllin, Trimethoprim, Sulfamethoxazole, Streptomycin, Tetracycline and Chloramphenicol (Nüesch-Inderbinen *et al.*, 2016). The antibiotic susceptibility tests clarify that *S. dysenteriae* has a wide range of resistance to many antibiotics. This may attributed to the presence of the extra outer cytoplasmic membrane which comprised of lipoproteins, lipid bilayer, lipopolysaccharides and polysaccharides. Furthermore, misuse or abuse of natural and synthetic antimicrobial agents could be part of the involvement factors of resistance to antibiotics (Mordi and Momoh, 2009). In both medical and

Table 1. Hemolysis pattern and protease production of *S. dysenteriae*

Isolates #	Hemolysis pattern on blood agar plates	Protease Production on skim milk agar
1	weak hemolysis	weak clear zone
2	weak hemolysis	weak clear zone
3	weak hemolysis	weak clear zone

Table 2. Hemaaglutination test for heated and non-heated supernatant of S. *dysenteries*

Dilution	Heated supernatant	Non-heated supernatant
1/10	No-lyses	Moderate
1/20	No-lyses	Moderate
1/40	No-lyses	Moderate
1/80	No-lyses	Moderate
1/160	No-lyses	Moderate
1/320	No-lyses	Moderate
1/640	No-lyses	Weak
1/1280	No-lyses	Weak

Table 3. The antibiotics susceptibility for *S. dysenteriae*

Antibiotics Disk	Disc conc. (µg)	Sensitivity of *S. dysenteriae* in (mm)	Results
Nalidixic acid	30µg	15	I
Tetracycline	30µg	1	R
chloramphenicol	30µg	10	R
ceftriaxone	30µg	26	S
co-trimoxazole	25µg	19	S
Ampicillin	10µg	2	R
ciprofloxacin	5µg	22	S

veterinary therapy, the source and period of microbial isolation, natural and the routine use of antibiotics may give rise to in wide prevalence of antibiotic resistance and development of antibiotic resistance genes particularly within the gram negative organisms. There are different mechanisms of antibiotic resistance include: decreased penetration of drug into the cell, efflux of antibiotics out the cell by efflux pumps, hydrolysis of antibiotics, and modification in target by mutation (Blair *et al.*,2015). The bla-TEM or bla-oxa genes are responsible for the development of ampicillin resistance, while resistance to chloramphenicol, streptomycin and tetracycline has mainly been attributed to the presence of catA1, strA and tetB genes respectively, but, the resistance to trimethoprim is associated with dhfrIa or dhfrIIIc genes (Toro *et al.*, 2005).The first drugs used to treat *Shigella* infections were sulphonamides and followed by tetracycline and then by chloramphenicol. *Shigella* spp developed their resistance to all of these drugs and finely treatment was shifted to ampicillin and co-trimoxazole. Today, ceftriaxone, azithromycin and pivmecillinam are recommended by WHO against infection caused by fluoroquinolones-resistant *Shigella* species (Taneja and Mewara, 2016). From all above variations in susceptibility, the antimicrobial agents are of great value for bequeathing curative measures against microbial infections. Whereas, gradually development in resistance to these agents is a significant cause of attention and periodic monitoring of drug resistance of these organisms should be carried out in different geographical areas so that appropriate agent can be chosen for empiric therapy. Both mammalian RD and L20B cell lines were exposure to heat and non-heated bacterial supernatant of *S. dysenteriae* respectively. The oscillatory manner of the cytotoxicity analysis was appeared within 18 hr. and the pH value was changed within less than 24 hr. after treatment with non- heated and heated of filtrated supernatant. Cell shape was shown changes include: seizure, shrinkage, segregation, rounding was observed as cytopathic effect. Cell death completely and lyses was notes with total destruction and caused either failure of cells in suspension to attach or detachment of cells from established monolayers within 120 hr. compared with non- treated cells as a control (Fig. 1). The pathogenesis of shigellosis or bacillary dysentery involve colonization, invade the epithelial cell in the lining of the colon, causing severe inflammation, due to *Shigella* cells grow and multiply intracellularly and spread to neighboring epithelial cells; after that ulceration , hemorrhage and tissue demolition were occurred in last stage of shigellosis is the death of the cells that lining the colon and fibrosis of the colonic mucosa region, all these symptoms associated with abdominal pain and this sore results in the severe bloody diarrhea and may contain mucus, or pus. For this, *Shigella* cells may cause dysentery (Kodati *et al.*, 2008; Hill Gaston, 2003). The abundance of extracellular secretion by *Shigella* spp has led to difficulty in characterizing these factors and to contravention in the enteropathogenicity. In general, cytotoxic the cell may produce severe illness in humans. These morphological changes can defined as cytopathic effects (CPE) and can be useful to classify cytotoxic, this damage may attributed together some factors such as **i)** Extracellular temperature – stable serine protease autotransporters (Sat) production to degrade components of extracellular matrix including collagens, laminin, elastin, and fibronectin, and finely cause the death of the cell after 120 hr. (Seshadri *et al*., 2006) **ii)** Ipa (IpaB and IpaC) protein secretion that insertion into RD and L20B cells membrane (Schiavolin *et al*., 2013) and **iii)** Shiga Toxin (an A-B toxin). Shiga Toxin causes cell death by preventing protein synthesis by cleaving a specific adenine residue from the 28s rRNA in the 60s subunit (Richardson, 1992). These explanations are compatible with the results of cytotoxic effect on RD and L20B caused by supernatant of *Aeromonas hydrophila* (Al-Rubai *et al.*, 2011).

There is no available studies refer to ability of *S. dysentria* to cytotoxic of RD or L20B cell lines, but there are many articles showed the cytotoxic effect of *Shigella* spp on other mammalian cell line. In vitro this organism has competence to infecting monolayers of Henle intestinal epithelial cells and HeLa cells line (Hale *et al.*, 1979) *S. flexneri* able to invade HeLa cells with high worthiness, This critical step in the pathogenic process is encoded by a 140 megadalton plasmid which induces phagocytosis of the bacteria by host cells. The virulent plasmid-associated hemolysin is a remarkable factor in

the invasion and amplification of *Shigella* spp. in different mammalian cells (Sansonetti *et al.*, 1986). The report by Brown and his colleagues explain that Shiga toxin produced by *S. dysenteriae* leads to inhibited protein and DNA syntheses in intact HeLa cells (Brown *et al.*, 1980). Also, *Shigella* is able to induce necrosis and apoptosis of host cells, the infected cells by *Shigella spp* or other dangerous pathogen bacteria rapidly lose membrane integrity due to the release of effector proteins such as cytoplasmic lactate dehydrogenase enzyme through the type III secretion system and the exposure of phosphatidylserine. The Interjection of a pore by *Shigella spp* into the infected host cell membrane result the necrosis (Nonaka *et al.*, 2003; Hersh *et al.*, 1999). Chen and his colleagues

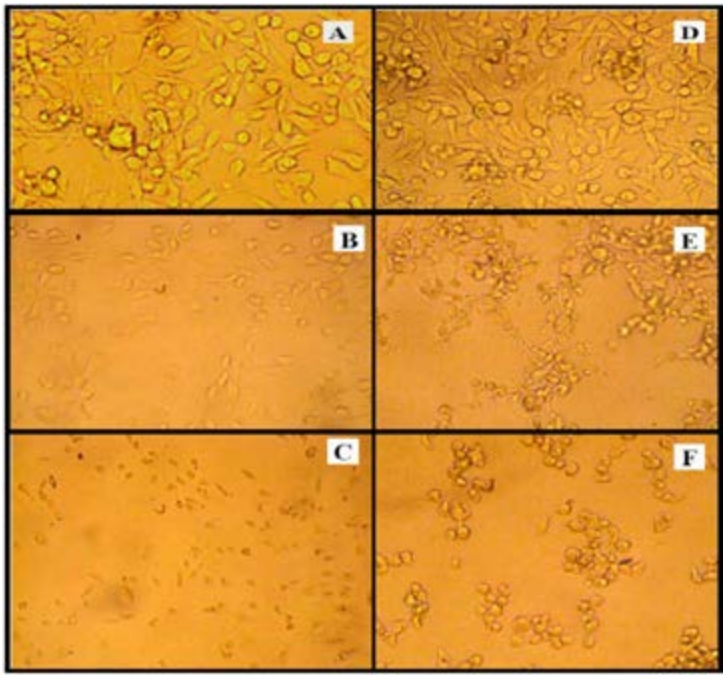

Fig. 1. The cytopathic action of *S. dysenteries* filtrate supernatant on mammalian cell lines were **A**: untreated RD cells **B**: RD cells treated with non-heated filtrate supernatant for 18 hr. the cells shown shrinking, detaching and rounded in shape **C**: degradation, lyses and most treated RD cells were death after treated with heated supernatant at 100°C after 120 hr. **D**: untreated L20B cells **E**: L20B cells shown shrinking, detaching, accumulation and rounded in shape after treated with non-heated filtrate supernatant for 18 hr **F**: death most treated L20B cells with heated supernatant at 100°C after 120 hr.

reported that releasing IpaB by *Shigella* lead to kill the macrophage in infected host. This factor directly binds to and activates caspase-1 to become as the key molecule in the induction of caspase-1-dependent apoptosis by *Shigella* infection (Chen *et al.*, 1996). In the growth of the variety of virulent strain of bacteria have been appeared ability to produce spherical Nan vesicles called outer membrane vesicles (OMVs), these vesical carry many toxins such as heat-labile toxins and other virulence factors include lipopolysaccharides (LPS), DNA or RNA, lipids, and proteins (Lee *et al.*, 2008; Kesty *et al.*, 2004).

These vesicles contain the same above factors that are known and contain other toxin such as Shiga toxin in OMVs of *E. coli* O157:H7 (Kolling .and Matthews, 1999), the cytolethal distending toxin in Campylobacter jejun vesicles (Lindmark *et al.*, 2009) and the Cif protein in OMVs of *Pseudomonas aeruginosa* (Bomberger *et al.*,2009). In our study can conclude that the haemolytic pattern by IpaB and IpaC and Sat activity of the *S. dysenteriae* might play an important role in the pathogenesis of the organism *in vivo* thus occurrence of the cytotoxicity. Although, the results appeared some correlation

between Sat activity and hemolysis with cytotoxic effects, may found other extracellular factors should be highlighted in relation to pathogenicity of *S. dysenteriae*. These factors might have diverse expression or under the same genetic control. It is not improbable that different virulence factors are serious under different event. These results clarify the presence of wide spread, multiple antibiotic resistant and cytotoxic *S. dysenteriae*. The linked between the hemolysis; proteolysis and cytotoxic activities must be further estimated to genetic control of other virulence factors, mechanisms genetically and network of these virulence factors in showed the pathogenesis of *S. dysenteriae*. ii) The destruction and damage of Both RD and L20B cell lines in our results by extracellular secretion produced by *S. dysenteriae* may effect on cancer cells and become weaker, the cytotoxicity of cancer cells are left alone vulnerable to attack by the active immune system and destroyed rapidly. Although the toxins of S. *dysenteriae* are dangerous, they can be employed to treat cancer cells as an alternative therapy or in the development of anti-tumor drugs through destruction of tumor mass.

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author declares that there is no conflict of interest.

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