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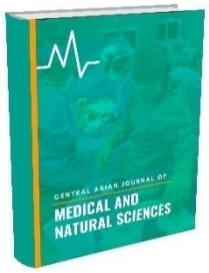

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The Effect of *Cronobacter Sakazakii* on the Gastro-Intestinal Tract of Newborn Mice

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Key words: Animal models, Cronobacter sakazakii, Gastro-intestinal tract, Mice, Newborn.

Abstract: Fifteenth of Cronobacter sakazakii were obtained from previous studies isolates were (5 infant formula, 5 spinal fluid and 5 bloods). C. sakazakii is ability to survive on long time in environment as powder infant material which leads transmission bacteria to the immune compromised infant . Fifteen new born mice were obtained from the Iraqi Center for Cancer and Medical Genetics whose ages ranged between 10-17 days and their weights 8.5 ± 1.25 gm, were divided into 3 groups. The samples were cut and placed in formalin, then placed in stabilizer and concentrations of alcohol (70%, 80%, 90%,100%) to complete dehydration after they were leached with xylene, embedded the samples paraffin wax (58°C) and sectioning the with thickness (5) micrometer and stained with Haematoxylin and Eosin stain. The results showed that swelled of the gastro-intestinal tract and fluid accumulation was observed for the animals vaccinated with C. sakazakii an increase in the weight of the gastro-intestinal tract for the mice compared to the control treatment and this increase was significant at the probability level $p \leq 0.05$ this may be due to the occurrence of inflammatory symptoms in the affected organ 72 hours after the injury the histological study of the gastro-intestinal tract and the tunica mucosa was distinguished by deep enterocytes goblet cells and muscular was thick . It was represented by loss of villi shedding of the epithelium with rupture of the intestinal epithelial cells and their rupture in the lower of the gastrointestinal tract. Edema of the sub mucosal epithelium has been observed. Goblet cells necrosis and filter immune cells neutrophils, macrophages, eosinophil, and lymphocytes fill the lumen C. sakazakii concentration 103 and 105 cells necrotizing enterocolitis of the newborn mouse.

Introduction:

The bottom consists of the lower gastro-intestinal canal in the mouse Tunica mucosa, Tunica sub mucosa, Tunica muscular and Tunica serosa contain lamina propria capillaries lymphatic vessels Lymphocytes and Plasma cell 1. Signs of necrotizing colitis in children are intestinal juices exudation vomiting flatulence, blood in stool and no rectal fissures were observed 2. adhesion to host surfaces intestinal epithelium, survival in the intestinal wall invasion and entry into the blood stream necessary process for infection formation 3. Cronobacter sakazakii is one of the member Enterobacteriaceae family, it is gram negative bacteria, rod-shaped, peritrichously flagellated and non-spore-forming, it is facultative anaerobic as it can growth without oxygen or grow with a small of oxygen, the growth temperature range is 6–45 °C with Optimum temperature of 37–43 °C 4 . C. sakazakii is ability to survive on long time in environment as powder infant material which leads transmission bacteria to the immune compromised infant 5. C. sakazakii caused-necrotizing enterocolitis in infants dangerous neurological diseases meningitis and septicemia 6 . The attack of bacteria to the host's cells is unknown but C.sakazakii have outer membrane proteins its help to affect the host's cells 7. Extracellular glycoproteins lead to adhesion intestinal epithelial and endothelial cells 8 .

Materials and Methods:

Fifteenth of C. sakazakii were obtained from previous studies. Isolates were (infant formula, spinal fluid and bloods). The isolate was inoculated more virulent depend on its speed of movement in the center of the MacConky agar at 37C for a period of 24 hours, after which a part was taken by loop in saline solution by McFarland standard solution 0.5 during dosing the newborn mice and their number was 15 obtaining healthy newborn mice from the Iraqi Center for Cancer and Medical Genetics whose ages ranged between 10-17 days and their weights 8.5±1.25 gm, were divided into 3 groups. The first group by the control treatment who were dosed with distilled water and the second group dosed with bacteria C. sakazakii (AIC) at concentration of 103cells/ml and the third group was dosed at concentration of 105 cells/ml .

Histopathological study

Tissue slides were prepared for all samples taken from newborn mice (colon samples). The samples were cut and placed in formalin, then placed in stabilizer and concentrations of alcohol (70%, 80%, 90%,100%) to complete dehydration and remove water from the tissue, after which they were leached with xylene, embedded the samples with a dissolved paraffin wax (58°C) and sectioning the with thickness (5) micrometer in transverse sections using rotary microtome and stained with Haematoxylin and Eosin stain, the sections were examined with a combined optical microscope equipped with a digital camera and taken Pictures using Live View Pro digital camera, directly from the computer 9,10.

Results:

The peritoneal cavity was opened the gastro-intestinal tract was examined with the naked eye, and swelling of the organ and fluid accumulation was observed for the animals vaccinated with C. sakazakii , also it was observed through the results of an increase in the weight of the gastro-intestinal tract for the mice compared to the control treatment and this increase was significant at the probability level $p \leq 0.05$ Fig.1. This may be due to the occurrence of inflammatory symptoms in the affected organ 72 hours after the injury the histological study of the gastro-intestinal tract using histological sections stained with hematoxylin-eosin stain control 11. The tunica mucosa was distinguished by deep enterocytes or Intestinal glands, goblet cells and circular muscle layer of the tunica muscular was thick Fig. 2 , 3. 12. It was represented by loss of villi shedding of the epithelium with rupture of the intestinal epithelial cells and disruption of the structural structure of the intestine and their rupture in the lower part of the gastrointestinal tract 13,14. Edema of the sub mucosal epithelium has been observed, goblet cells necrosis and filter immune cells neutrophils, macrophages, eosinophil, and

lymphocytes fill the lumen C. sakazakii concentration 103 and 105 cells necrotizing enterocolitis of the newborn mouse involved most of the linings of the intestinal wall Fig. 4, 5, 6 , 7 15, 16.

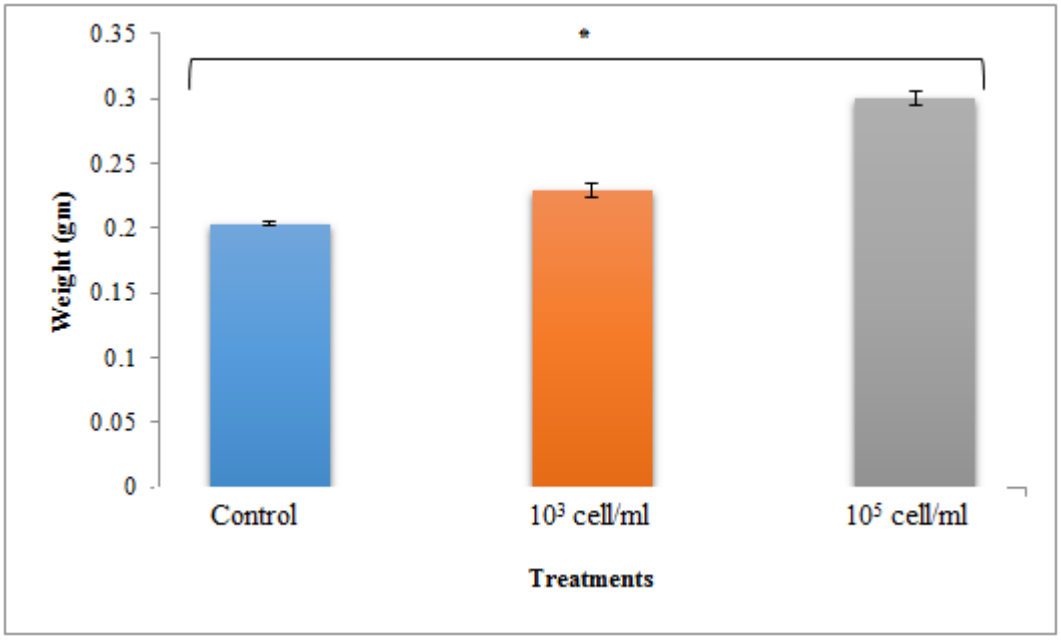

Figure 1: Changes in the gastro-intestinal tract weight of newborn mice dosed with C. sakazakii Compared with the control treatment, there are significant differences under the probability level $p \leq 0.05$.

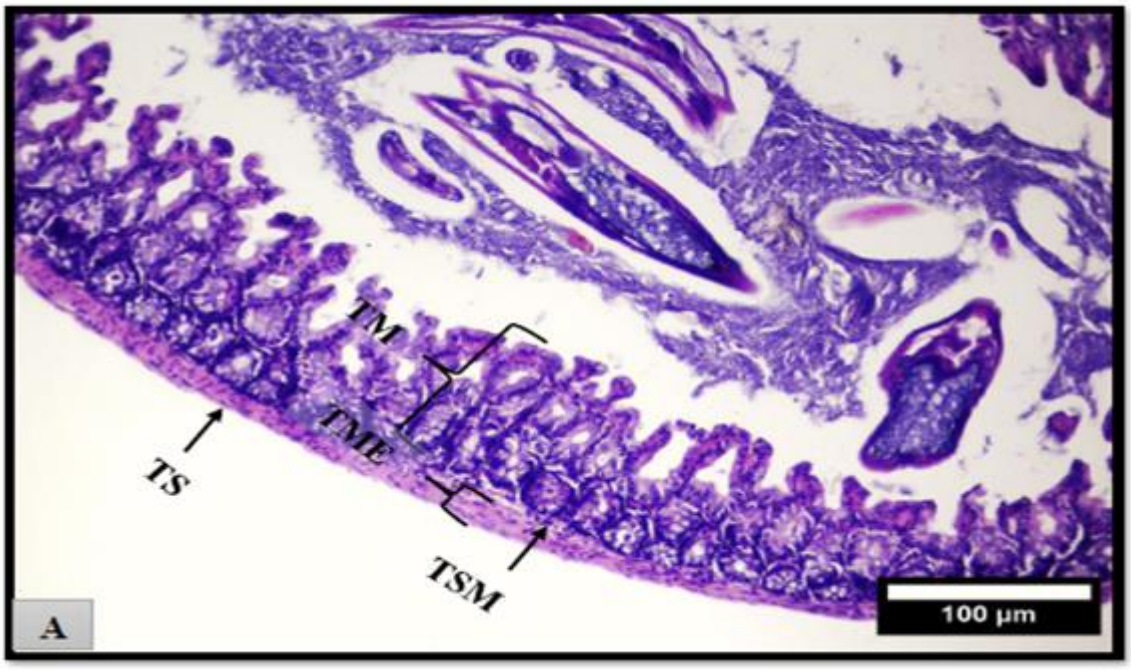

Figure 2: Cross-sectional section in the lower part of the gastro-intestinal tract of newborn rat of the control group, note: tunica mucosa (TM), tunica submucosa (TSM), tunica muscle (TME), serous tunica (TS), (hematoxylin-eosin stained, A-strength 10x)

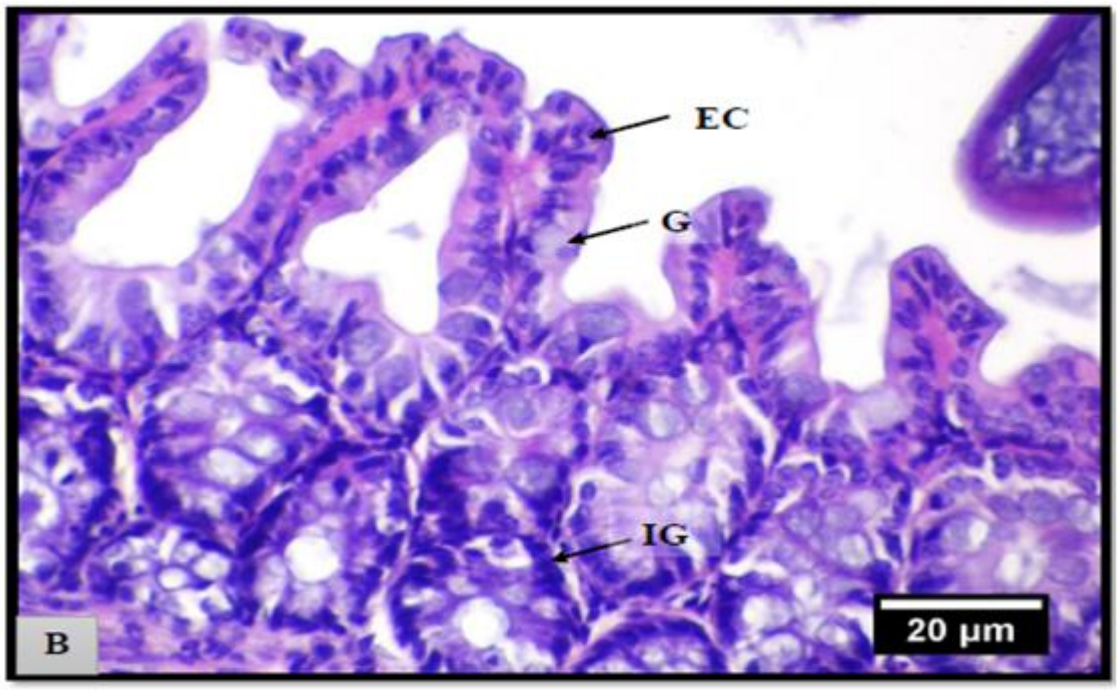

Figure 3: Cross-sectional section in the lower part of the gastro-intestinal tract of newborn rat of the control group, note: enteric glands (IG), enteric cells (EC), goblet cells (G) , (hematoxylin-eosin stained, B-strength 40x).

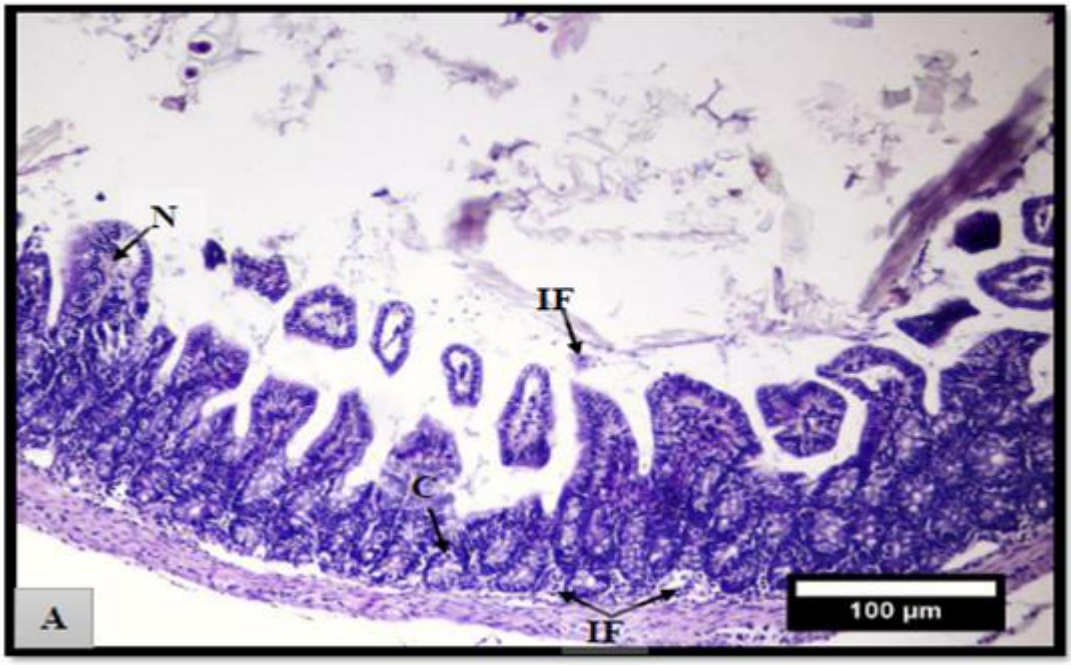

Figure 4: Cross- section in the lower part of the gastro-intestinal tract of newborn mouse inoculated with *C. sakazakii* concentration of 103 cells/ml, note: intestinal rupture (IG), infiltration of immune cells (IN), mucosal necrosis (N) (TME), edema (O), (hematoxylin-eosin stained, A-strength 10x).

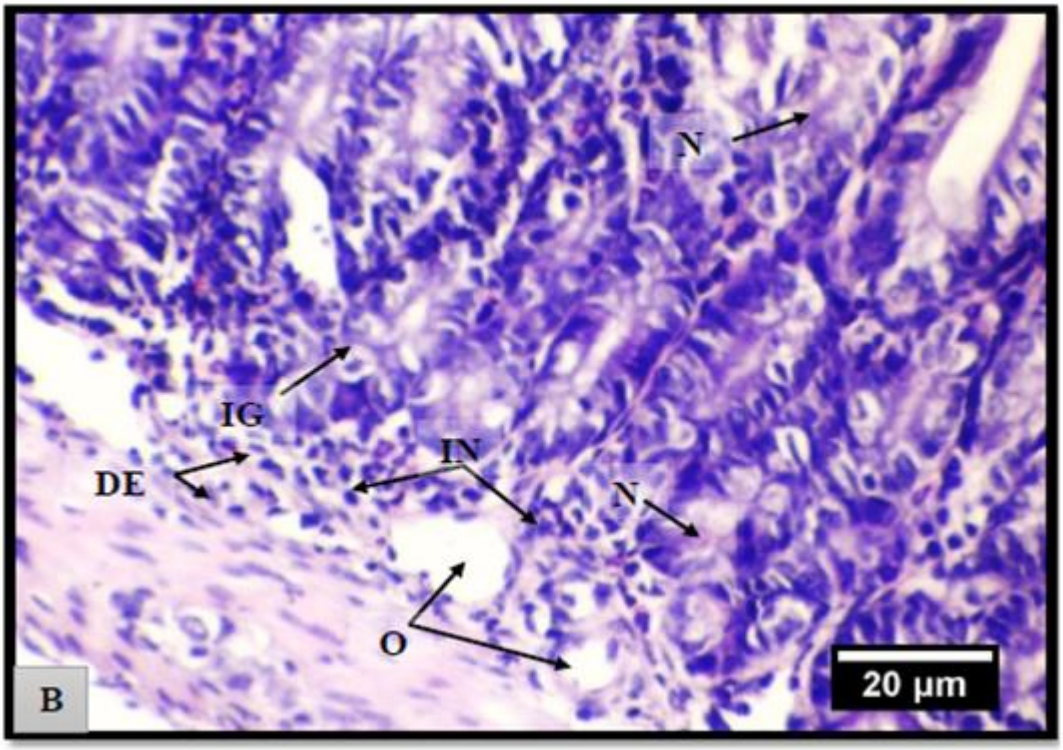

Figure 5: Cross- section in the lower part of the gastro-intestinal tract of newborn mouse inoculated with *C. sakazakii* concentration of 103 cells/ml, note: intestinal rupture (IG), infiltration of immune cells (IN), mucosal necrosis (N) (TME), edema (O), (hematoxylin-eosin stained, B-strength 40x).

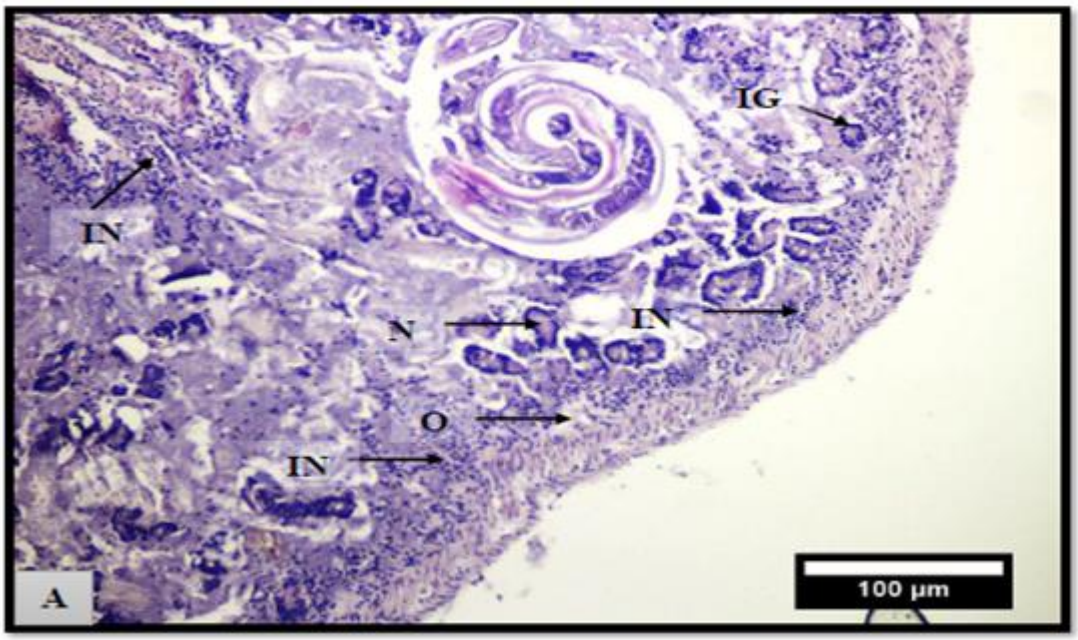

Figure 6: Cross - section in the lower part of the gastro-intestinal tract of a newborn mouse inoculated with *C. sakazakii* at a concentration of 105 cells/ml, note: necrosis (N), , intestinal rupture (IG) and degeneration (DE), Immune cell filtering (IN) and edema (O), (hematoxylin-eosin stained, A-strength 10x).

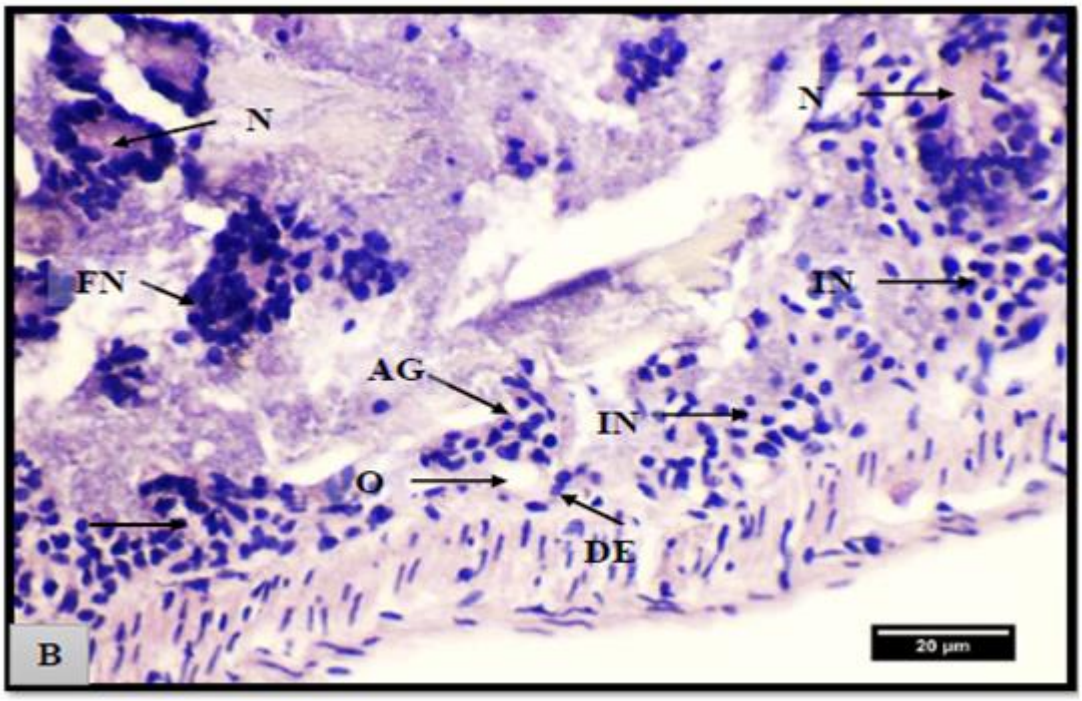

Figure 7: Cross - section in the lower part of the gastro-intestinal tract of a newborn mouse inoculated with *C. sakazakii* at a concentration of 105 cells/ml, note: necrosis (N), focal necrosis (FN) of the entirety forming layers of the intestine, degeneration (DE), Immune cell filtering (IN) and collecting (AG), edema (O), (hematoxylin-eosin stained, B-strength 40x).

Discussion:

C. sakazakii attaches, invasion and multiplies in the intestinal cells of the gastro-intestinal tract and causes these damages responsible for the appearance of necrotizing enterocolitis , the damage caused by these bacteria in the tissue of the intestinal wall may be due to the presence of many bacteria C. sakazakii in the gastro-intestinal tract which caused damage to the tissue wall of the gastric duct and was a clear cause of the emergence of the inflammatory immune response 11. Intestinal inflammation caused by C. sakazakii infection may be associated with several damaged and dead intestinal tissue cells resulting from infection 17. Bacteria on the intestinal tissue and cause is necrotizing enteritis it is due to its possession of LPS, leads to an increase in the permeability of intestinal epithelial cells, leads to the transmission of bacteria 18.

The ability of C. sakazakii to invasion intestinal epithelial cells is due to the presence of the genes Ffli and tn5, proteins that encode the movement of bacteria have a major role in the process of cell invasion 19,20. C. sakazakii of environmental or nutritional origin have the ability to migrate intra cellular after the invasion process due to their ability to overcome all physical barriers of the intestine 21. Presence omp A of a role in the injury of intestinal necrosis through a change permeability and apoptosis enterocytes 22.

Conclusion:

From the current study, it can be concluded that C. sakazakii bacteria has the ability to cause weight changes in newborn mice and pathological weight and tissue of important organ in the mouse body (gastro-intestinal tract) when these mice are dosed at different concentrations of C. sakazakii..

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.

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