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The Eeffect of *Cronobacter sakazakii* on the Brains of Newborn Mice

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Abstract

Fifteenth of *Cronobacter sakazakii* were obtained from previous studies isolates were (5 infant formula,5 spinal fluid and 5 bloods). 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 an increase in the weight of the brain organ of the treated mice compared to the Control and this increase was significant at the probability level $p \leq 0.05$ and the results showed the inflammatory response of the brain of large number of polymorphnuclear cells and mononuclear cells as well as congested blood vessels with an increase in the size of endothelial cells , presence of bleeding and shrinking of nerve cells in the the cerebral cortex gliosis (neuroma), was more prominent in the molecular layer in the brain of the newborn mice fed at a concentration of 10^5 cells and the occurrence of oedema and presence of haemorrhage in both concentrations of dosed newborn mice and necrosis of the choroid plexus concentration10^3 and 10^5 cells.

Keywords: *Cronobacter sakazakii*, Brain, Animale models.

1. Introduction

The brain and spinal cord are the central nervous system and are surrounded by the meninges, and the mouse brain is composed of cerebral cortex, basal ganglia, hippocampus, thalamus, hypothalamus, cerebellum and brain stem. The cerebrum consists of hemispheres divided into (frontal, parietal, temporal and occipital lobes). Meninges is the connective tissue that rounds the brain and spinal cord made up dura mater arachnoid and pia mater [1]. *Cronobacter sakazakii* is one of the Enterobacteriaceae family, it is gram negative bacteria, rod-shaped, peritrichously flagellated and non-spore-forming , it is facultative anaerobic as it can grow without oxygen or grow with a small of oxygen, the growth temperature range is 6-45°C with an optimum temperature of 37-43°C [2]. *C. sakazakii* can survive for a long time in the environment as powder

infant material which leads transmission of bacteria to the immune compromised infant [3]. OmpA , help to break blood-brain barrier and invasion the central nervous system causing meningitis [4]. *Cpa* gene encodes outer membrane proteins for a role in serum resistance, that enables *C. sakazakii* to cross the blood-brain barrier and cause meningitis [5]. Some studies inside the body of the living organism have shown *Cronobacter* invasion of the brain cells , especially the microvascular endothelial cells of the human brain and the brain cells of both rats and mice [6]. The study aimed to investigate the histological effects of *C. sakazakii* on the brain of newborn mice .

2. Materials and Methods

2.1. Identification of isolates and inoculated

Fifteenth of *C. sakazakii* were obtained from previous studies were (5 infant formula, 5 spinal fluid and 5 bloods). The isolate was inoculated more virulent depending on its speed of movement in the canter of the MacConky agar at 37°C 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 number was 15 obtaining healthy newborn mice from the Iraqi Canter 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 was the control treatment who which were dosed with distilled water , the second group was dosed with bacteria *C. sakazakii* AIC (Dialak) at a concentration of 10^3cells / ml and the third group was dosed at a concentration of 10^5 cells / ml .

2.2. Histopathological study

Tissue slides were prepared for all samples from newborn mice (brain samples). The samples were cut and placed in formalin, then put in stabilizer and concentrations of alcohol (70%, 80%, 90%,100%) to complete fixed then 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] micrometre 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 conducting data analysis using the statistical program SPSS (Inc., Chicago, IL, USA) [6], [7], [8].

3. Results

The skull was opened, the brain 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 brain organ for the mice compared to the control treatment and this increase was significant at the probability level $p \leq 0.05$ conducting data analysis using the statistical program SPSS (Inc., Chicago, IL, USA) (**Figure 1**).This may be due to the occurrence of inflammatory symptoms in the affected organ72 hours after the injury the histological study of the brain using histological sections dyed with hematoxylin-eosin stain (9). Natural meninges membrane was pia matter formation thin layer loose connective tissue outer surface simple squamous epithelium tissue as for inner surface pia matter arachnoid adjoining nervous tissue of the brain and found white, gray membrane oligodendrocytes to hippocampus and choroid plexus was cuboidal epithelium molecular layer neuropil neuron cell bodies (**Figure 2 and 3**). Showed the inflammatory response of the brain showing the presence of a large number of polymorphnuclear cells and mononuclear cells as well as congested blood vessels with an increase in the size of endothelial cells, also acute inflammation of the brain was observed represented by the presence of foci of inflammatory cells most of which are neutrophils cells in the meninges concentration 10^3 and 10^5 cells (**Figure 4 and 5**). This may be because of the due to the fact that acute inflammation leads to pleocytosis which is an essential sign of meningitis and the presence of bleeding congestion and shrinking of nerve cells in the area of the cerebral cortex, which also appears in the cerebral gliosis (neuroma), was more prominent in the molecular layer in the brain of the newborn mice fed at a concentration of 10^5 cells (**Figure 6**) and the occurrence of oedema, Spotty neuronal necrosis (**Figure** 7), which was the result of increased vascular permeability in

the cerebral cortex region
effective lymphocytes and microglia were also observed and associated with the presence of

haemorrhage in both concentrations of dosed newborn mice and necrosis of the choroid plexus concentration 10^3 and 10^5 cells (**Figure** 8 and 9).

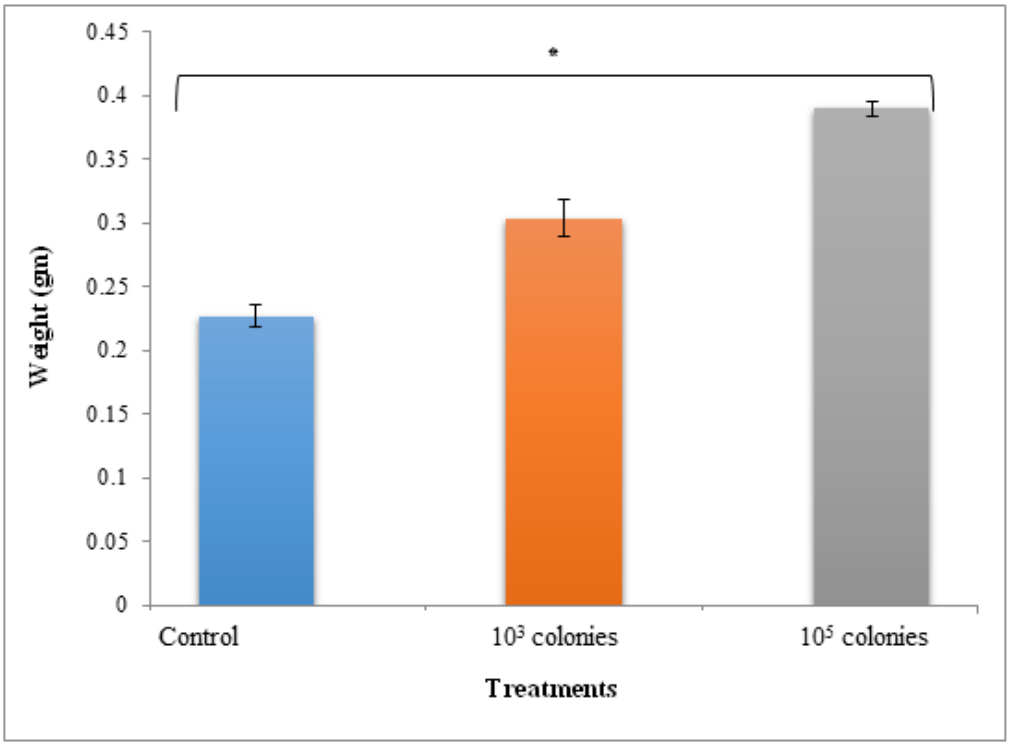

Figure 1. Changes in brain weight of newborn mice dosed with C. sakazakii Compared with the control group. The results revealed a statistically significant difference at $p<0.05$.

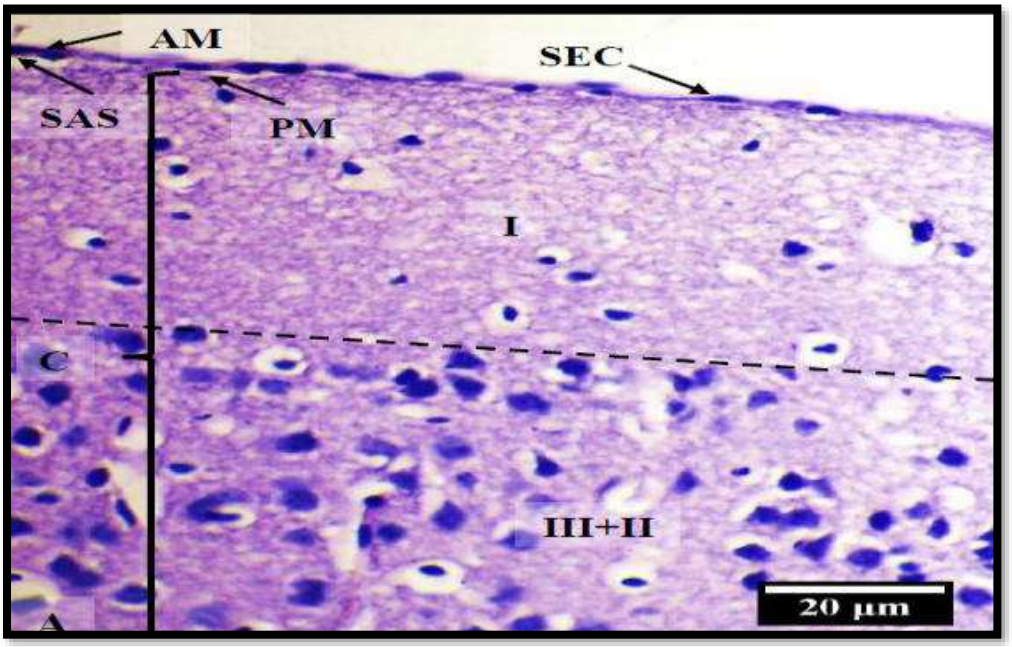

Figure 2. Section of the brain of a neonatal mouse (Cerebrum) of a control group note: arachnoid envelope (AM), squamous epithelial cells (SEC), cortex (C), layers I, II and III cortex (I and III+II) subarachnoid spaces (SAS), pia (PM), (hematoxylin -eosin stained, 10x, A: 40x power).

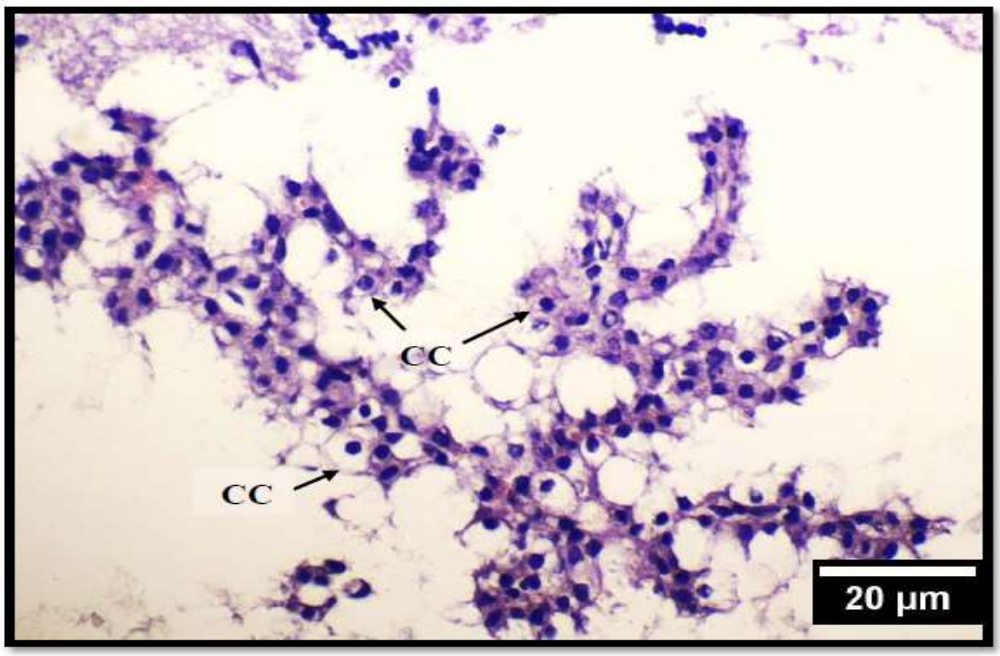

Figure 3. Cross section of the brain of a newborn mouse of the control group showing a region of choroid plexus note: cuboidal epithelial cells (CC), stained with hematoxylin -eosin, 40x power.

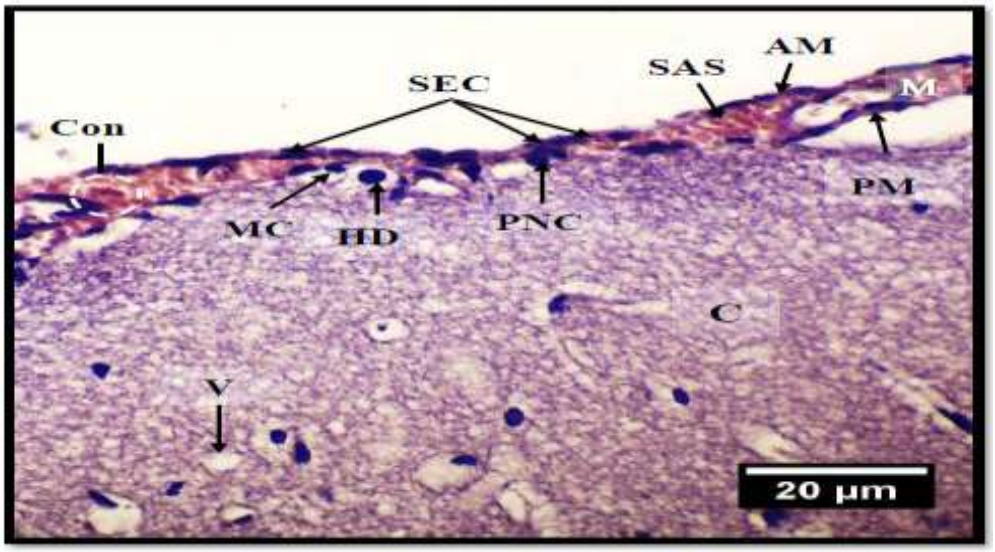

Figure 4. Brain of newborn mice (Cerebellum) inoculated with *C. sakazakii* at a concentration of 10^3 cells, note: arachnoid envelope (AM), squamous epithelial cells (SEC), cortex (C), subarachnoid space (SAS), pia (PM), monocytes nucleus (MC), polymorphonuclear cells (PNC), congested blood vessels (Con), clonal degeneration (HD), ventricular (V) stained with hematoxylin-eosin,40x power.

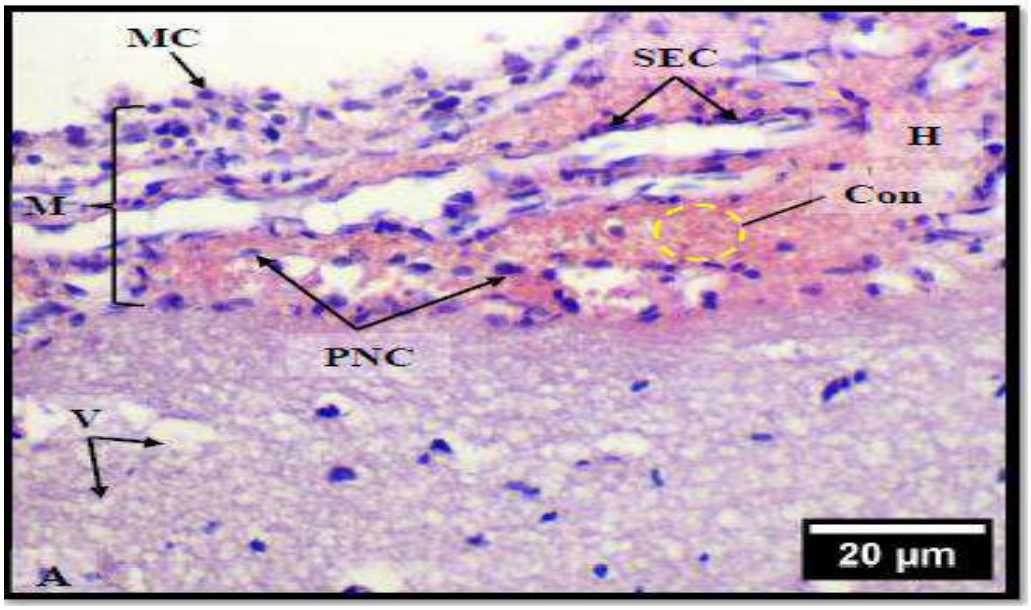

Figure 5. Cross section of a newborn mice (Cerebrum) inoculated with *C. sakazakii* at a concentration of 10^5 cells/ml. Note: Mononuclear cells (MC), nucleus (PNC), hemorrhage (H) and congested blood vessels (Con) lobed cells, stained with hematoxylin-eosin,40x power.

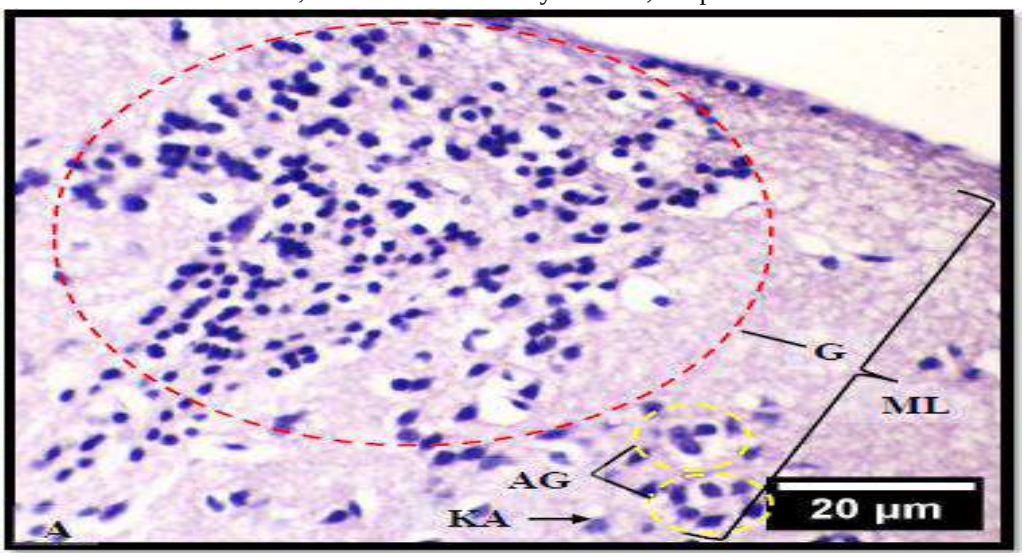

Figure 6. Cross section of the brain of mice *C. sakazakii* concentration of 10^5 cells/ml note: the glioma (G) in the molecular layer (ML) of the cortex area ,nucleolysis (KA), and aggregated neurons (AG), stained with hematoxylin-eosin,40x power.

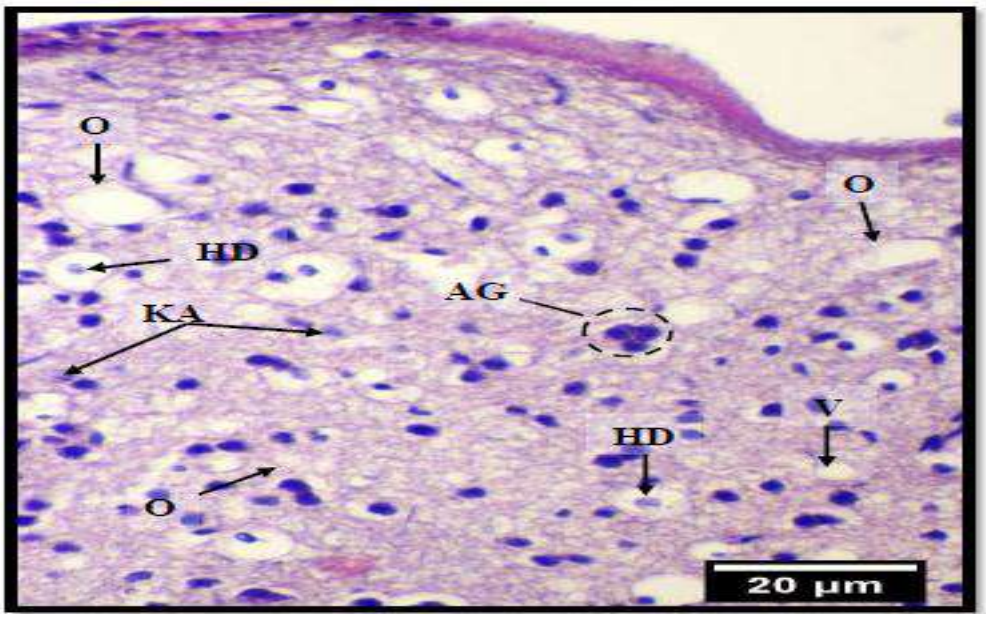

Figure 7. Cross section of the brain of a newborn rat dosed with *C. sakazakii* concentration of 10^5 milligrams/ml, note: edema (O), edema (HD), edema (V), nucleus bearing (KA), nucleoli hypertrophy (HT), neuronal pool (AG), stained with hematoxylin-eosin,40x power.

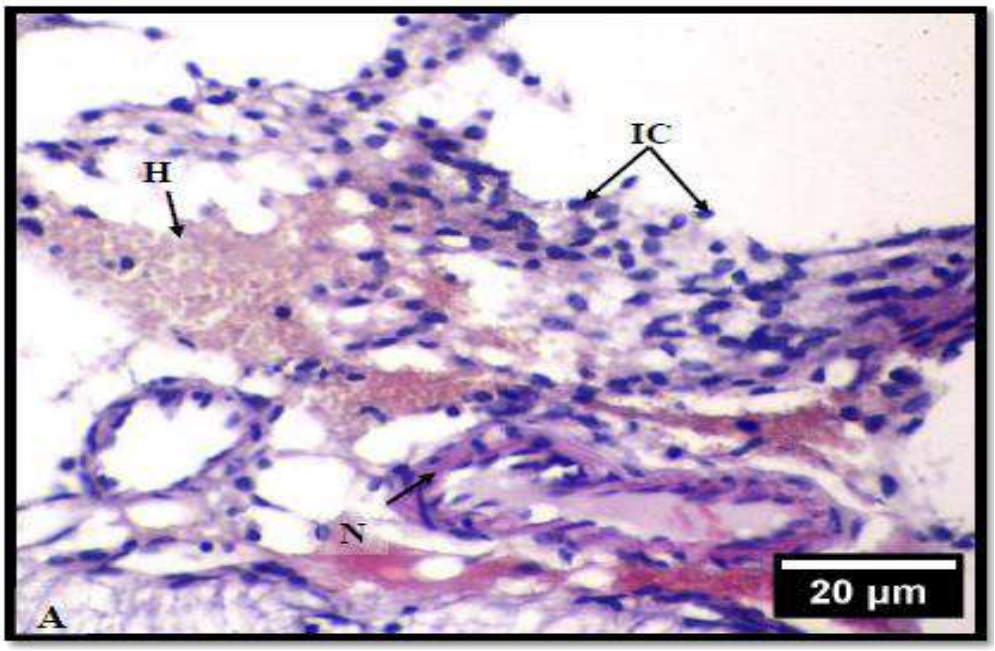

Figure 8. Cross section of the choroid plexus of the brain of a newborn mice *C. sakazakii* at a concentration of 10^3 cells/ml, note: inflammatory cells (IC) and necrosis (N), hemorrhage (H), stained with hematoxylin-eosin,40x power.

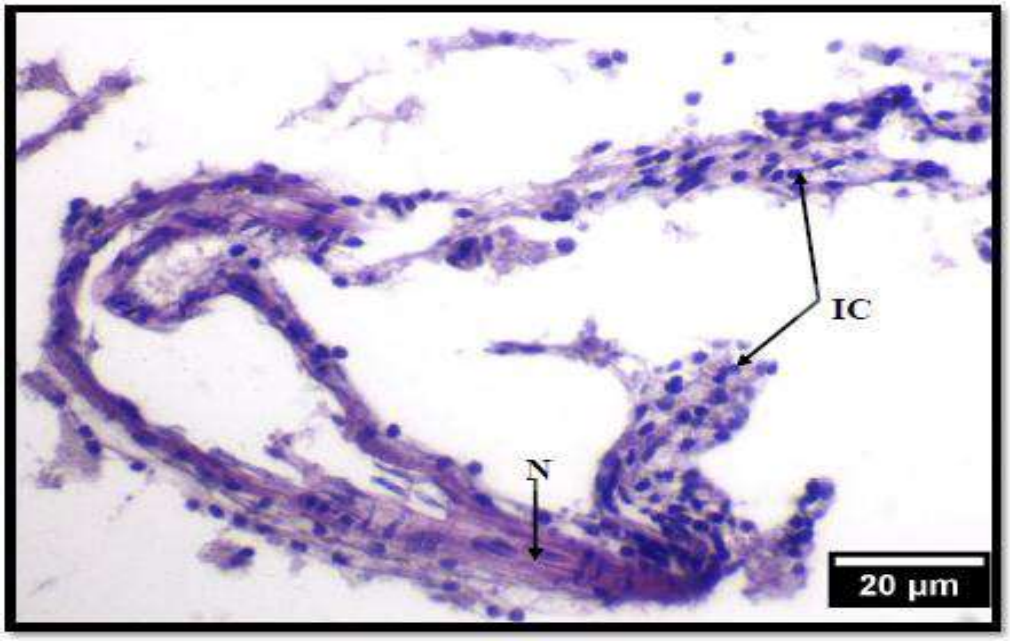

Figure 9. Section of the choroid plexus of the brain of a newborn mice inoculated with *C. sakazakii* at a concentration of 10^5 cells/ml, note: inflammatory cells (IC), necrosis (N). scale bar fixed on the photo

4. Discussion

This may be because acute inflammation leads to a large number of cells of the cerebrospinal fluid cell, an important sign of meningitis. In this study, reported the infection of mice orally dosed with *C. sakazakii* with meningitis by crossing the blood brain barrier in newborn mice reported *C. sakazakii* dosed in mice caused severe inflammation of the choroid plexus area [10]. It was confirmed that infection with *C. sakazakii* bacteria causes inflammation and tissue changes in the brain [11]. The association of these multiple inflammatory foci within the brain with the blood vessels as the bacteria move into the space rounding the blood vessels indicates that *C. sakazakii* may enter the cerebrospinal fluid causing a massive influx of inflammatory cells into the brain's ventricles and meninges and rupture intercellular communication enters the cerebral parenchyma edema may as a result of increased permeability of blood vessels in the cortex, neuronal cell death and cell release of hydrolase causes liquefaction and necrosis of the brain results in ischemic damage probably to edema and pressure (decreased neuronal density and liquefaction within the cortex) [12]. When they studied mice orally dosed with *C. sakazakii* the occurrence of gliosis in the cerebral cortex due effect of this bacteria on mice *C. sakazakii* dosed It caused inflammation symptoms similar to what happened to the studied mice in the area of the cortex and the substance white [13]. *zpx* gene encode to proteolytic enzymes to break intravenous collagen (IV) cause destroys the endothelial cell membranes of blood vesselsdestruction this leads to the leakage of blood components into the tissues enables bacteria to cross through blood brain barrier [14].These changes may be to bacterial such as lipopolysaccharides Lipopolysaccharides or other toxins secreted by bacteria or the virulence factor *OmpA* gene is required to adhesion and invasion endothelial cells in the brain In one of the necessary steps during the development of meningitis [15], [16].

5. Conclusion

From the current study, it can be concluded that *C. sakazakii* can cause weight changes in the brains of the treated newborn mice. Also, histopathological changes were detected in this vital organ of the mice inoculated with different concentrations of *C. sakazakii*.

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