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RESEARCH ARTICLE OPEN ACCESS

Comparative study of brain and eye infected by *Escherichia coli* Isolated From River Water in Male and Female Rats

No sex differences in the Histology of E. coli-infected Rat Cerebrum

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Abstract

Objective: One important hormone and neurotransmitter that is vital to the body and brain is dopamine. It functions as a chemical messenger between neurons in the brain as a neurotransmitter, impacting several critical functions like mood regulation, reward and pleasure systems, motor control, and cognitive function. A varied group of bacteria called *Escherichia coli* (*E. coli*) (STEC strain) is frequently found in both human and animal intestines. Although the majority of *E. coli* strains are benign and essential for gut health and digestion, certain strains can seriously cause harm to individuals. There is a lack of information regarding shiga toxin-producing *Escherichia coli* (STEC) in river water, and it is crucial to comprehend its true situation.. This bacterium can infect and cause changes in behaviour and daily life. Therefore, rats (males and females) were infected to determine the changes that might occur between them.

Methods: Ten rats (five male and five female) were infected with *E. coli,* and brain samples were collected to make histological sections and assess dopamine levels.

Results: This bacterium infects the brain through the eyes and affects the cerebral histologic and dopamine level with *significant* differences.

Conclusion: Our findings show that an *E. coli* infection changes histological brain and dopamine levels, pointing to a possible connection between gut flora and neurological function, and there are no differences between male and female.

Keywords: Escherichia coli, Eye, Brain, Dopamine, Histology

Plain English Summary

One important hormone that is important to the human body and brain is dopamine. It is a chemical messenger in the brain that affects critical functions like mood regulation, motivation and movement. *Escherichia coli* (*E. coli*) may influence dopamine pathways. This study aimed to assess the histological comparison of the infected cerebrum by *Escherichia coli.* In male and female rats. Ten rats (5 male & 5 female) were infected with *E. coli,* and brain samples were collected to make histological sections and assess dopamine levels. This study's findings showed that *E. coli* infection changes brain dopamine levels, pointing to a possible connection between gut flora and neurological function.

Background

Brain disorders, including meningitis and cerebral vasculitis, can be caused by a wide variety of microorganisms. Escherichia coli is one of these bacteria, which are extra-intestinal pathogens that may spread to several tissues beyond the gut and

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result in dangerous diseases like meningitis. According to recent in vitro and in vivo studies, *E. coli* strains can infiltrate the brain, and the most frequent way this bacterium develops in the brain is through infection-induced disruption of the brain blood barrier (BBB).

One of the central nervous system's (CNS) most important defences, the blood-brain barrier (BBB), is vital for preventing the transmission of pathogens (1, 2). Although pathogenic strains of Escherichia coli (*E. coli*) are typically associated with gastrointestinal disorders, they can also have serious neurological effects, especially in severe cases. This association is directly explained by the bacterium's ability to produce toxins, induce systemic inflammation, and, in rare cases, enter the central nervous system (CNS). In a previous study, serum dopamine levels were compared among thirteen male and female rats; there were significant differences between male and female rats' dopamine concentrations.

E. coli is a member of the Enterobacteriaceae family of bacteria and one of the most notable diseases. It is the most prevalent in commensal inhabitants of the gastrointestinal tracts of humans and other warm-blooded animals (3). In light of its commensal connection, which is mutually beneficial, it seldom spreads disease. However, because it causes a variety of ailments, it is also one of the most common infections in both humans and animals (4). Neuronal growth, cognitive regulation, mental state, emotional regulation, behaviour, and brain function are all significantly impacted by the gut-brain axis, a bidirectional communication system that connects the gut microbiota to the brain (5, 6).

One of the most common neurotransmitters, dopamine, concentration in the brain is directly correlated with how well the brain functions (7). Dopamine is an important neurotransmitter in the reward system, motivation, pleasure, and motor activity. As a chemical messenger, it transmits signals between nerves and is important in many bodily and mental activities like regulation, learning, and movement. It is made from the amino acid tyrosine and is mainly produced in the substantia nigra and ventral tegmental area of the brain (8, 9).

Materials and methods

In this work, *E. coli* (STEC) was obtained from the Al-Hilla river of Al-Hillah city located in the province of Babylon, Iraq. The target of pathogenic genes of *E. coli* (STEC) in this investigation were stx1 and stx2, which are associated with the generation of Shiga toxin. Additionally, a particular gene found in E. coli called udiA was added. Both pathogenic genes of *E. coli,* stx1,stx2 and udiA, were detected by the PCR method (10). *E. coli* (STEC) was injected into rats' eyes to infiltrate their brains for a month. The animals were then dissected to remove the eyes and brain for histological sectioning and to homogenise each one.

Isolation of Bacteria

Bacteria were isolated by using the standard membrane filtration technique (USEPA, 2000) (1). The water sample was collected from Al-Hilla river samples collected from the river by dipping the bottle, neck down, below the surface while holding it close to the base in the hand. Serial dilution was made (10-1 to 10-5), ready and mixed thoroughly. 10 ml of water sample was transferred to 90 ml of normal saline that filtered through a cellulose acetate membrane sterile 0.45 µm filter using a vacuum filtration system, and transferred cellulose filter paper was transferred to chromogenic agar and incubated at 37 °C. Each sample was examined in triplicate.

Animals

The animals were housed in a controlled environment (37°C) with unlimited access to food and water. Every attempt was made to decrease animal suffering and the number of animals utilised.

Anaesthesia and Procedures

For all invasive operations, animals were sedated with Isoflurane at adequate doses to provide complete sedation and analgesia.

Twenty rats (10 male and 10 female), 5 male and 5 female infected with *E. coli* through the eye, and the other group was the control. Control rats were given regular saline, and no alterations were seen (11, 12). All animals were dissected to obtain eye and brain tissue, The autopsy was undertaken when it was determined that the injury had certainly happened in the eye, as seen in Figure 1. Each sample was cut into two parts (one was kept in 4% formaldehyde for the brain).



Figure 1: Lab rats: A. Rats before being infected with Escherichia coli appear with normal eyes; B, C and D: Infected rats with Escherichia coli after one month of infection show infected eyes

Histological section
All sections were soaked in alcohol at different concentrations and later sectioned and stained with hematoxylin and eosin. These sections were examined at 40x.

Dopamine
Dopamine levels were determined in homogenised samples using ELISA kits (Elabscience).

Results
All animals lost their appetite for food for four days, became lethargic and immobile, and some rats died during that period. According to the histology section, male control and *E. coli-infected* samples varied in the structure of their cerebrum tissue. Sections reveal brain tissue with cerebral oedema, microthrombosis, associated with ischemic changes, neuronal shrinkage and few mononuclear inflammatory cells, are shown in Figure 2 (B). The tissue structure in (A) is more consistent and preserved, indicating that histopathological alterations have been brought on by bacterial infection in (B). Figure 2 shows Sections from this investigation that show brain tissue with cerebral oedema, microthrombosis, ischemic alterations, neuronal atrophy, and a few mononuclear inflammatory cells. There was evidence of neuronal injury in the tissue, indicating that histopathological alterations may have been caused by bacterial infection. figure 3 B shows a well-preserved neuronal architecture and a rather homogenous organisation. The lack of notable histological alterations. The tissue was normal in section A, Figure 3.

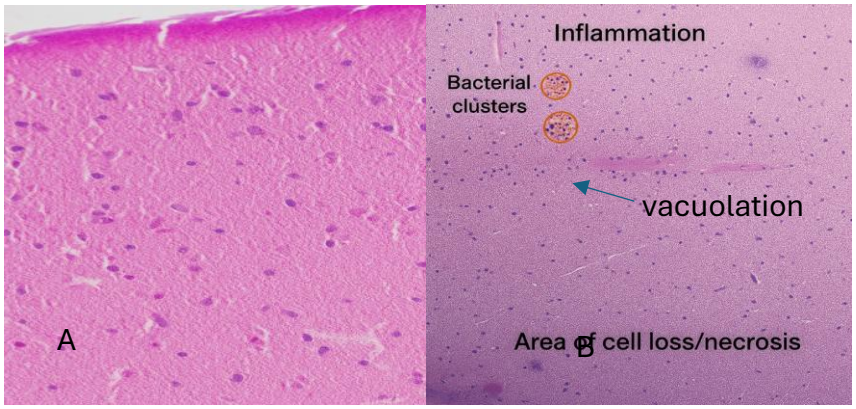


Figure 2: longitudinal section of cerebrum male, A: normal cerebrum, B: infected cerebrum H&E, 40x

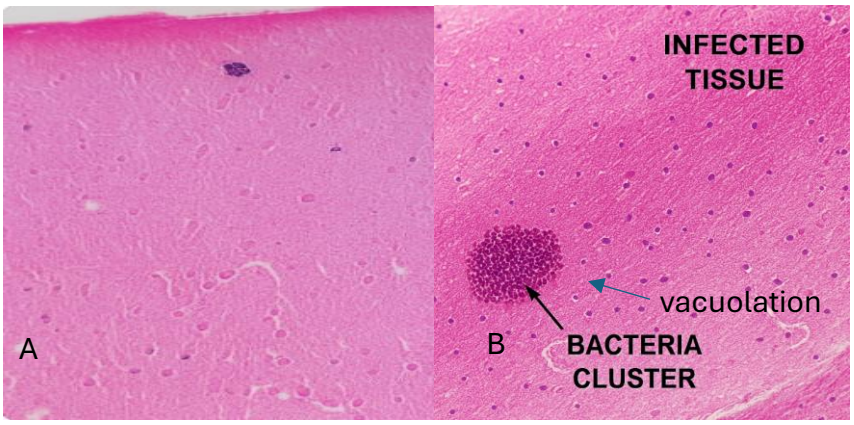


Figure 3: longitudinal section of cerebrum female, A: normal cerebrum, B: infected cerebrum, H&E, 40x

Males (50.6040 ± 0.82729) and females (50.8340 ± 0.45941) had similar levels of dopamine in the brain. There was no statistically significant difference in the dopamine levels in the male and female brains, according to the p-values for both groups (0.814 and 0.816). In the eye, dopamine levels in males (5.09902) and females (164.0680 ± 5.07505) differ significantly. Although there was a substantial difference in the mean values between the male and female groups, the p-value (0.795) indicated that the variation may not be statistically significantly different (Table 1).

Table 1: Differences in dopamine among normal brains and eyes for males and females

Group		Mean ± SD(ng/mL)	P - value	Cohen's d
brain	Female	50.8340±.45941	.814	≈ -0.13
	Male	50.6040±.82729	.816	
eye	Female	164.0680±5.07505	.795	≈ 0.088
	Male	163.0670±5.09902	.795	

* *Non-Significant Differences P > 0.05*

Female brain dopamine levels were 63.3200 ± 3.03547, whereas male brain dopamine levels were 64.3400 ± 3.85505, with a p-value of 0.841. Dopamine levels in the male and female brains did not differ significantly. In female eyes, dopamine levels were 99.4720 ± 5.03418, which was in contrast with those of the male eyes (96.6560 ± 11.24727) with a p-value of 0.827. The dopamine levels in the eyes of males and females do not differ significantly. There is no statistically significant difference between male and female dopamine levels in either the brain or the eyes, according to the p-values (> 0.05). The overlapping standard errors indicated that there was a large degree of variability within each group, despite the small numerical difference (Table 2).

Table 2: Differences in dopamine among infected brains and eyes for males and females

Group		Mean ± SD(ng/mL)	P-value	Cohen's d
Brain	female	63.3200±3.03547	.841	-0.131
	male	64.3400±3.85505	.841	
Eye	female	99.4720±5.03418	.825	0.145
	male	96.6560± 11.24727	.827	

Non-Significant Differences P > 0.05

Male Brain Dopamine Levels in Infected Males: 64.34 ± 8.62 vs. Control: 50.60 ± 1.85 P-value: 0.02 => Dopamine levels in infected males were statistically significantly higher than those in controls. Also, dopamine levels in infected females were significantly higher than those in controls (63.32 ± 6.79 versus 51.14 ± 1.18 in the control group; P-value: 0.01). Dopamine levels in Infected female eyes: 99.47 ± 11.26 and control: 164.07 ± 11.35. P-value: 0.00 => Dopamine levels in infected females were significantly lower than those in controls. Males infected had significantly lower dopamine levels than controls (96.66 ± 25.15 vs 166.00 ± 12.34714), with a P-value of 0.02.

Infected males and females had higher brain dopamine levels than controls. The brain's generation of dopamine may be impacted by an *E. coli* infection, according to the significant p-values (0.02 and 0.01). In contrast to controls, dopamine levels dropped considerably in the infected eye groups. The *E. coli* infection had a considerable impact on lowering dopamine in the eye, as indicated by the highly significant p-values (0.00 and 0.02) (Table 3).

Table 3: Differences among brains and eyes for males and females after being infected with bacteria

Group		Mean ± SD(ng/mL)	P - value
Brain male	Infection	64.3400 ± 8.62015	0.016
	Control	50.6040 ± 1.84989	
Brain female	Infection	63.3200 ±6.78752	0.01
	Control	51.1400±1.18237	
Eye female	Infection	99.4720±11.25676	0.00
	Control	164.0680± 11.34815	
Eye male	Infection	96.6560± 25.14966	0.016
	Control	166.0000± 12.34714	

Significant differences P < 0.05

Discussion

According to the study's findings, an *E. coli* infection dramatically changes the brain's dopamine levels. During the early stages of some bacterial infections, immunological activity may activate the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased dopamine turnover in the mesolimbic pathway (13, 14). Some bacteria, including gut-resident and opportunistic infections, may generate or modify dopamine and other neuroactive chemicals. For example, E. coli strains can create biogenic amines such as dopamine or its precursors (tyrosine, L-DOPA). This microbial production can boost local or systemic dopamine levels, particularly when gut permeability is weakened (15). Bacterial endotoxins, especially lipopolysaccharides (LPS), have been found to enhance dopamine metabolism and cause neuroinflammation by inducing microglial activation (16). Furthermore, histological analysis of infected brain tissues showed structural variations, confirming the hypothesis that harmful bacteria might weaken the integrity of neurons.

The retina's amacrine cells generate dopamine, which is essential for visual processing and circadian rhythms. During infections, bacterial endotoxins lower retinal dopamine levels, resulting in diminished visual sensitivity and poor adaptation to light, as evidenced in animal models of diabetic and inflammatory retinopathies (17).

The gut-brain axis is a bidirectional communication system that connects the gastrointestinal tract with the central nervous system (CNS) via neuronal, hormonal, and immunological signalling pathways. The gut microbiota plays an important role in controlling this axis, and disturbances can affect mood, behaviour, and cognition (18). According to some research, these strains can indirectly influence brain function by regulating short-chain fatty acid (SCFA) synthesis and the availability of neurotransmitter precursors such as serotonin and dopamine (19). Dopamine synthesis starts with

tyrosine, and while E. coli does not manufacture dopamine in significant quantities; it can Microbial-host nutritional interactions compete for or affect the availability of tyrosine and other amino acid precursors, respectively. Influence other bacterial communities that generate dopamine-like chemicals (e.g., Bacillus and Lactobacillus), changing the neurochemical balance. Furthermore, E. coli may create biogenic amines such as tyramine and cadaverine, which can affect host neurophysiology and the autonomic nervous system (15).

These results highlight the need for more investigation into the role that pathogenic bacteria play in neurological diseases and support the mounting evidence of a gut-brain link. Future research should investigate the long-term effects of bacterial infections on neurodegeneration, as well as possible treatments that target microbial pathways.

Conclusion

Our findings show that an *E. coli* infection changes brain dopamine levels, pointing to a possible connection between gut flora and neurological function. Future research should investigate the underlying molecular processes, such as the interactions of bacterial endotoxins with brain circuits. New therapies for neurological conditions initiated by the bacteria may be feasible if these interactions are well-understood.

List of Abbreviations

E.coli: Escherichia coli
BBB: Blood-brain barrier
CNS: Central nervous system

Declarations

Ethical approval and consent to participate

All animal operations followed the ethical norms of the University of Babylon. Before the start of the investigation, the Academic Research Ethics Committee of [University of Babylon] examined and approved the research protocol under permission number [Z240303].

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

Nil.

Funding

Nil.

Author contributions

AHM: Draft.Data Curation, Formal Analysis, Visualisation; HRSH: Conceptualisation, Methodology, Writing the Original Draft; AZM: Investigation, Resources, Writing, Review and Editing; MRK: Supervision, Project Administration, Funding Acquisition.

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