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EFFECT OF CAFFEINE ON BLOOD CULTURE AND GASTROINTESTINAL MICROFLORA OF ALBINO RATS

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Abstract

The present study aimed to evaluate the microbiological impact of caffeine administration on the gastrointestinal microflora and systemic infection risk in albino rats. The experiment was designed to determine whether caffeine exposure could compromise immune status or increase susceptibility to bacteremia. Male albino rats were administered three doses of caffeine citrate (3 mg, 6 mg, and 9 mg), calculated from typical human intake and adjusted for body weight, while untreated animals served as controls. Blood cultures were performed to assess the presence of bacteremia, and fecal samples were analyzed to determine alterations in gut microbial composition. No bacterial growth was detected in the blood samples of either treated or control animals, indicating the absence of bacteremia and no evidence of systemic infection. The gastrointestinal microbiota of treated animals remained largely similar to that of controls, with Escherichia coli, Lactobacillus spp., and Staphylococcus aureus representing the predominant flora. A slight increase in Bacillus subtilis was observed in animals receiving the highest caffeine dose, but this change did not indicate major disruption of microbial balance. Overall, the findings suggest that the tested caffeine dosages did not compromise immune integrity, nor did they significantly alter normal gut microflora in healthy albino rats.

Keywords: *Albino rats, Blood culture, Caffeine, Gastro-intestinal tract, Microflora (B. subtilus), Pathogenic bacteria*

INTRODUCTION

Caffeine is a methyl xanthine alkaloid found in seeds, leaves and nuts of a number of plants in East Asia and South America. Caffeinated soft drinks and energy drinks have their main effects on the brain (1). The anti-inflammatory effects of caffeine are due to nonselective competitive inhibition of phosphodiesterases (PDEs). It fights temporary fatigue and enhances mental focus, which leads to better concentration and mood. The beverages with caffeine are associated with healthier and more diverse community microorganisms in the gut without a major impact on dominant microbes (2). The effect of caffeine on the population and diversity of microbes of the large intestine of adult Wistar rats was studied (3). The results showed that caffeine use changed the diversity and population of microbial species. In the gut, which may influence the health of the consumer?

The Guarana (*Paullinia cupana* Mart.), which is a medicinal plant with high caffeine compounds, also negatively affected gut microorganisms in Wistar rats (4). A study revealed that the use of energy drinks containing caffeine in youngsters results in negative health impacts (5).

The present study investigated the caffeine effect on the blood culture GIT microflora of albino rats. The immediate aim of the present work was microbiological evaluation of the caffeine effect on blood and fecal matter. The dosages were designed in such a way as to correspond with those of human beings.

MATERIALS AND METHODS

All procedures were performed in agreement with guidelines of committee of ethics on animal use by Vertebrate Pest Control Institute, University of Karachi (No.1998401).

ADMINISTRATION OF DRUG DOSAGES

Caffeine citrate (Lahore Pharma) was used throughout the studies as a source of caffeine. Three different dosages were selected and administered to the rats. The dosages were calculated to be equivalent to the amount of caffeine present in the drug Cafergot® (i.e. 100 mg). The dosages were calculated according to the weights of the rats in such a concentration, to match the dosages given in combination with Cafergot® to the animals. The dosages are given in Table I.

Table I. Drug dosage of caffeine given to test rats and its regimen

S. No.	Animal group (3 rats each)	Dosages (mg)	No. of days	Mean weight of rats
1	C_1	15	90	200 gm
2	C_2	30	90	200 gm
3	C_3	45	90	200 gm
4	C_4	-	90	200 gm
5	CA	125	90	200 gm

C_1: Rats treated with 15 mg of caffeine, C_2: 30 mg of caffeine, C_3: 45 mg of caffeine, C_4: Rats given no caffeine simply distilled water which served as control, CA: Rats treated with 125 mg/kg

MICROBIOLOGICAL STUDIES ON EFFECT OF CAFFEINE ON TREATED ANIMALS

In order to check if the animals became immuno-compromised the treated animals were continuously monitored for any abnormalities. A regular blood culture was done to check bacteremia. The microbiological detection was done according to Wistreich and Lechtman (1988). Fecal samples were also microbiologically analyzed to detect any pathogenic bacteria. The cultures used for blood samples were Nutrient agar and Blood agar. Nine agars were used for the fecal samples the microbes have vastly different environmental for proper growth, thus, for comprehensive coverage different media were used namely Nutrient agar (E & O Laboratories Ltd.), Blood agar (Oxoid), MacConkey's agar (E & O Laboratories Ltd.), Eosin methylene blue agar (Titan Biotech. Ltd), Brain heart infusion agar (Oxoid), Sabouraud Dextrose agar (E & O Laboratories Ltd.), Salmonella shigella agar (Criterion), Bismuth sulfite agar (Criterion) and Skimmed milk agar (Chemsolute). The agar plates were incubated for 24th at room temperature (range 37-42° C) in the Laboratory of Microbiology Department, University of Karachi, Karachi-75270.

RESULTS

The microbiological analysis of blood test for control and treated animals revealed no bacteria growth i.e. no bacterium was detected and the blood sample of the treated animals was similar to the control (Table II).

Likewise, feces of the treated animals did not show presence of any pathogenic bacteria and the microbial contents were the same as for the control (untreated) except for *B. subtilis* which increased in Brain heart infusion agar for rats treated with acute dose of caffeine (125 mg) (Table III). The gram-negative bacteria isolated were *Escherichia coli* and the gram-positive bacteria isolated were *Lactobacilli* sp., *Staphylococci aureus* and *Bacillus subtilis.* Cfu per g normal values (+) for *E. coli* ranges 10^4 to 10^6 cfu/g feces, *Lactobacillus* 10^4 cfu/g feces, *S. aureus* 10^3 g/feces and *B. subtilis* 30^8g/feces while higher value (++) for *B. subtilis* was 30^8-30^9 g/feces.

Table II. Mean values of microbiological findings of blood samples of rats treated with different doses of caffeine

	Animal groups					
Media	Caffeine *C_1	*C_2	C_3	*C	Acute doses *CA	Organism isolated
NA	-	-	-	-	-	Nil
BA	-	-	-	-	-	Nil

C_1: Rats treated with 15 mg of caffeine, C_2: Rats treated with dose of 30 mg of caffeine, C_3: Rats treated with dose of 45 mg of caffeine, CA: The animal group were given acute dose of 125 mg of caffeine, C: Control animals were given distilled water, NA: Nutrient agar, BA: Blood agar, -: Negative, no growth, *: There were three replicates for each observation

Table III. Mean values of microbiological findings of fecal samples of rats treated with different doses of caffeine

Media	Caffeine		Rats groups			Organism isolated
	*C_1	*C_2	C_3	*C	Acute doses *CA	
NA	+	+	+	+	+	*E. coli* *Lactobacilli* sp.
BA	+	+	+	+	+	*S. aureus*
Mac	+	+	+	+	+	*E. coli*
EMB	+	+	+	+	+	*E. coli*
BHI	+	+	+	+	++	*B. subilis*
SDA	-	-	-	-	-	Nil
SSA	-	-	-	-	-	Nil
BSA	-	-	-	-	-	NIL
SMA	+	+	+	+	+	*Lactobacilli* sp.

C_1: The Rats treated with dose of 15 mg of caffeine, C_2: The Rats treated with dose of 30 mg of caffeine, C_3: The Rats treated with dose of 45 mg of caffeine, CA: The Rats treated with acute dose of 125 mg of caffeine, C: Control animal were untreated, NA: Nutrient agar, BA: Blood agar, Mac: MacConkey's agar, EMB: Eosin methylene blue agar, BHI: Brain heart infusion agar, SDA: Sabouraud dextrose agar, SSA: Salmonella shigella agar, BSA: Bismuth sulfite agar, SMA: Skimmed milk agar, -: Negative, no growth, +: Positive, present, ++: Increased growth observed, *:There were three replicates for each observation

In all treatment groups, the composition of the intestinal microbiota remained largely unchanged compared to the control animals, indicating that oral administration of caffeine at the tested doses did not disrupt major bacterial populations in the gut. The consistent isolation of *E. coli*, *Lactobacilli spp.*, and *Staphylococcus aureus* across control and experimental groups suggests that caffeine did not exert a broad-spectrum antimicrobial effect in vivo. This stability in the bacterial profile demonstrates that the gastrointestinal ecosystem of healthy rats exhibits resilience to the levels of caffeine administered in this study.

However, growth of *Bacillus subtilis* was notably increased on Brain Heart Infusion (BHI) agar in the group receiving an acute high dose of caffeine (125 mg). This selective increase may indicate that *B. subtilis*, a spore-forming organism capable of surviving environmental stress, could have a growth advantage under conditions modified by elevated caffeine exposure. Since other bacterial species showed no measurable change, the response appears species-specific rather than representative of a general shift in microbial equilibrium. This further emphasizes that caffeine's microbiological impact may depend on the physiology and metabolic capabilities of individual bacterial taxa.

No fungal growth was detected in any group, as evidenced by the absence of colonies on Sabouraud Dextrose Agar (SDA). Additionally, selective enteric media including Salmonella Shigella Agar and Bismuth Sulfite Agar showed no growth, indicating that neither pathogenic enteric bacteria nor fungi emerged in response to caffeine exposure. Together, these findings suggest that caffeine did not predispose animals to opportunistic or pathogenic colonization within the gastrointestinal tract. The microbiota remained within normal physiological ranges, underscoring that the administered caffeine doses, even at high levels, were not sufficient to induce dysbiosis in otherwise healthy rats.

DISCUSSION

Caffeine and other energy-boosting beverages have gained widespread popularity in recent years. Cho *et al.* 2018 reported that Americans consume an average of 200 mg of caffeine daily (5). In the present study, acute administration of caffeine did not produce significant alterations in the fecal microbiota of treated animals, except for a slight increase in the population of Bacillus subtilis in fecal samples.

Furthermore, microbiological examination of blood samples revealed the absence of bacteria, indicating that bacteremia did not occur. This suggests that caffeine did not compromise the immune defenses of the animals or increase their susceptibility to systemic infection.

Our findings contrast with those of Nawrot *et al.*, 2003 who demonstrated that caffeine significantly inhibited the growth of *Escherichia coli* (6). However, the results were more consistent with those of Al-Janabi *et al.*, who reported that caffeine did not alter the growth rate of *Proteus mirabilis* (7). These differences support the idea that caffeine's antimicrobial effects may be species-specific.

Cowan *et al.* 2014 found that coffee consumption in male Sprague Dawley rats fed a high-fat diet resulted in reduced body weight, decreased liver triglycerides, lower tissue adiposity, and decreased energy intake (9). Additionally, coffee increased the Firmicutes-to-Bacteroidetes ratio and elevated Clostridium cluster XI, typically associated with high-fat feeding, along with increased levels of Enterobacteria. These findings indicate that several microbiome changes may be attributed to coffee intake rather than caffeine alone.

Hegde *et al*. 2022 demonstrated that treatment with both coffee and decaffeinated coffee reduced intestinal microbial counts in vivo without significantly affecting gut motility or smooth muscle contractility (10). However, in vitro studies showed that both elicited ileal and colonic muscle contractions via muscarinic receptor–dependent pathways, suggesting that microbial alteration occurred independently of caffeine.

In a mouse model of metabolic syndrome, Nishitsuji *et al.* 2018 observed a significant increase in Gram-positive bacteria and a reduction in Gram-negative bacteria following coffee consumption (14). Age-related sensitivity to caffeine, such as ventricular arrhythmias, has also been described in older rats by Chen *et al.* 2022 (11).

Similarly, *Song et al.* reported that while Firmicutes and Bacteroidetes populations remained stable in caffeine-treated mice, Actinobacteria and Proteobacteria were markedly reduced, indicating that caffeine may influence microbial abundance and metabolic function (12). In the present study, however, neither Actinobacteria nor Proteobacteria were detected.

Scorza *et al.* 2022 observed that caffeine used as a common adulterant in cocaine samples significantly increased the Firmicutes-to-Bacteroidetes ratio in rats chronically exposed to volatilized cocaine (13). This further supports the notion that caffeine can modulate the intestinal microbiota under certain conditions.

Overall, previous studies indicate that caffeine may function as a selective antimicrobial agent, with its effects varying among microbial species. The present findings contribute to this growing body of evidence by demonstrating that caffeine can influence certain intestinal bacterial populations without promoting systemic infection or weakening the host's immune defenses.

CONCLUSION

Although *Bacillus subtilis* is generally regarded as non-pathogenic to humans and animals, it may still cause adverse effects such as nausea, vomiting, pneumonia, or septicemia in immunocompromised individuals. Therefore, the findings underscore the importance of clear and accurate labeling of caffeine content on energy drink products, ensuring that consumers,particularly vulnerable populations,can make informed and safe choices.

Conflict of interest:

There is no conflict of interest in this study.

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Authors' contribution:

NMG Performed the experiments; AK Provided technical outputs and wrote the manuscript; SAS Provided relevant literature; SND Provided some agars; AKh Proof reading

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