EFFECTS OF CAFFEINE AND NICOTINE ON THE POPULATION AND DIVERSITY OF GUT BACTERIAL MICROBIOME OF ADULT WISTAR RATS

Ojezele, Matthew Obaineh[1*]; Ehwarieme, Daniel Ayobola[2]; Atube, Mercy[1] and Potokiri Augustina[1]

[1]Department of Pharmacology and Therapeutics, Delta State University, Abraka, Delta State, Nigeria.
[2]Department of Microbiology, Delta State University, Abraka, Delta State, Nigeria.
*Corresponding author. E-mail: matlar2002@gmail.com. Tel:+2348033923332.

Accepted 24 October, 2017

The study was conducted to evaluate the effect of nicotine and caffeine on the population and diversity of microbiome of the gut (large intestine) of adult Wister rats. Seventy-seven (77) adult Wistar rats weighing 150 to 200 g were randomly grouped into eleven (11) groups of seven rats each. Group 1 served as control, received water which was the vehicle (group 2 (caffeine 10 mg/kg), group 3 (caffeine 20 mg/kg), group 4 (nicotine from cigarette 170 mg/kg), group 5 (nicotine from cigarette 340 mg/kg), group 6 (nicotine from tobacco 170 mg/kg), group 7 (nicotine from tobacco 340mg/kg), group 8 (caffeine 10mg/kg and nicotine from cigarette 170 mg/kg), group 9 (caffeine 20 mg/kg and nicotine from cigarette 340 mg/kg), group 10 (caffeine 10 mg/kg and nicotine from tobacco 170 mg/kg), group 11 (caffeine 20 mg/kg and nicotine from 340 mg/kg)). The agents were administered each day, orally for 14 days. On the fifteenth day, chloroform anaesthetized animals were sacrificed after which the large intestine were harvested, homogenized and microbial populations cultured to assay for changes in population and diversity of the microbiome of the gut (large intestine). Results obtained showed significant increase in the bacteria population of animals administered caffeine 20 mg/kg, nicotine from cigarette 170 mg/kg, nicotine from cigarette 340 mg/kg, nicotine from tobacco 170mg/kg, caffeine 20mg/kg and nicotine from cigarette 340 mg/kg, caffeine 10 mg/kg and nicotine from tobacco 170 mg/kg as compared with the control. *Bacillus* sp. was present in all groups except the groups administered caffeine 10mg/kg and nicotine from cigarette 170mg/kg. *Pseudomonas* sp. was only observed in the group administered caffeine 20mg/kg. *Staphylococcus* sp. however was present in the control but was absent in the group administered caffeine 10mg/kg and nicotine from cigarette 170mg/kg, and the group administered caffeine 20mg/kg and nicotine from tobacco 340mg/kg. This study has shown that nicotine and caffeine consumption can alter the population and diversity of microbial species in the gut. This may likely influence the general health status of the consumer. Further studies in this regard are recommended.

Key words: Microbiome, Wistar rats, caffeine, nicotine.

INTRODUCTION

Caffeine occurs naturally in nature as an alkaloid, found in plants in varying quantities. It can be found in beans and quarana berry. Caffeine is consumed worldwide from a range of dietary sources such as tea, coffee, cocoa beverages chocolate bars, soft drinks and more recently energy drinks. An estimated, 80% humans globally consume products containing caffeine on a daily basis (Karch, 2009), amounting to about 120, 000 tons of caffeine per annum (Ted and Norman, 2004).

Caffeine is a central nervous stimulant which belongs to the methylxanthine family, and acts by antagonizing adenosine receptor subtypes (A1,A2A,A2B,A3 receptors) there by promoting neurotransmitter release (for example, monoamines and acetylcholine) which gives it's stimulant effects (Fisone et al., 2004). Caffeine is mainly consumed for its energy giving properties; it temporary fights fatigue and enhances mental focus leading to improved mood and concentration. It is included in many over-the-counter medications because it has the ability to relieve allergy and headache. It has both natriuresis and diuretics properties which

are mediated via proximal tubular adenosine receptor blockade (Armstrong et al., 2007).

Nicotine is a cholinergic receptor agonist which is an alkaloid belonging to the nightshade family of plants (Malenka et al., 2009). *Nicotiania tabacum* is a herbaceous plant which grows annually. Its leaves are grown in several countries where it is processed into tobacco. There are two forms of tobacco; combustible tobacco (cigarette smoke) and smokeless tobacco (snuff). The World Health Organization (WHO) evaluated that tobacco intake will be the largest single health challenge by 2020, causing about 8.4million deaths every year (Viano et al., 2001). In United States alone, the death of 430,000 people each year is due to tobacco use. The mortality rate is about 5million every year and this number is still on the rise (Garret et al., 2001). Nicotine works as both a stimulant and a relaxant. Nicotine consumption stimulates the release of several chemical messengers including endorphin; the body's natural analgesic. Intake of nicotine promotes concentration and memory; it also helps to reduce pain due to the analgesic effect of endorphin.

Microbiome or microbiota is defined as a group of microbes or microorganisms that live in an environment. The human microbiome is composed of communities of commensals, symbiotics, and pathogenic bacteria (along with fungi and viruses), these entire microorganisms see the human body as home. A major focus of the human microbiome research has been the study of bacteria in the gut, both in terms of population (abundance) and diversity. Gut microbiome reflects the largest community. In humans, the gut flora has the largest population of bacteria, and has the highest diversity when compared to other parts of the body (Quigley, 2013). The gut microbiota in human beings changes and evolves throughout life, and plays a prominent role both in physiologic and diseased conditions. It changes when diet changes, and also when overall health changes (Rakaoff-Nahoum et al., 2016).

Drinking of coffee, tea, and other beverages containing caffeine is associated with healthier and more diverse community of microbes in the gut (Jaquet et al., 2009). In a complicated biocycle of caffeine, it is utilized by bacteria species such as *Pseudomonas* and by-product of their metabolism such as ammonia which it's then processed by nitrifying microorganism to nitrate. It further utilizes nitrates to sustain growth (Dash and Gummadi, 2006), apparently leading to significant changes in the population and diversity of species of the gut bacteria.

Nicotine has been shown to have effect on the population and diversity of species on the oral cavity of people taking nicotine (smoking), mostly on the periodontal tissue (Karina *et al.*, 2008). Bacteria such as *Pseudomonas* species, *Klebsilla* species, and *Bacillus* species are found to be the more effective in utilizing nicotine (Krishma and Thangavel, 2016; Biedermann et al., 2013).

In most communities in Nigeria, some individuals smoke cigarette, some chew tobacco while others inhale tobacco (snuff). These same individuals may consume products containing caffeine such as cocoa beverages, soft drinks, tea, coffee, chocolate bars and kolanut. This study is geared towards finding the impact of caffeine and nicotine on the population and diversity of bacteria of the gut microbiome (large intestine). It is hoped that this study would provide useful/additional information on the health implication involved in caffeine-nicotine combination that is to say, microbiome alterations.

MATERIALS AND METHODS

Extraction and drug preparation

Using distilled water as solvent, nicotine from cigarette and tobacco leaves was extracted using soxhlet extraction method. The filtrate was concentrated with the aid of a water bath at 40°C, thereafter; the concentrate was stored refrigerated prior to use, as previously described by Pavia et al. (1976) with slight modifications.

Drugs, reagents and chemicals

Nicotine was obtained from cigarette (B & H®) purchased locally. Tobacco leaf was sourced locally and identified at Department of Botany, Delta State University, Abraka, Nigeria. Caffeine was obtained from Karmel (CAS number: 10026-22-9). All reagents are analytical grade. The final doses of caffeine were 10 and 20 mg/kg (Adebayo et al., 2007), nicotine doses were 170 and 340 mg/kg (Biala and Weglinsa,

2004). All agents were administered orally once daily to the test animals.

Study design

The study was conducted on 77 adult Wistar rats weighing between 150 to 200 g. The animals were acclimatized for 14 days, fed with pure water and feed devoid of either nicotine or caffeine, after which they were randomly divided into 11 groups of 7 animals each, and were grouped as follows:

Group 1 served as control (Administered distilled water as vehicle)
Group 2 (Caffeine 10 mg/kg)
Group 3 (Caffeine 20 mg/kg)
Group 4 (Nicotine from cigarette 170 mg/kg)
Group 5 (Nicotine from cigarette 340 mg/kg)
Group 6 (Nicotine from tobacco 170 mg/kg)
Group 7 (Nicotine from tobacco 340 mg/kg)
Group 8 (Caffeine 10 mg/kg and nicotine from cigarette 170mg/kg)
Group 9 (Caffeine 20 mg/kg and nicotine from cigarette 340 mg/kg)
Group 10 (Caffeine 10 mg/kg and nicotine from tobacco 170 mg/kg)
Group 11 (Caffeine 20 mg/kg and nicotine from tobacco 340 mg/kg).

The agents were administered for 14days.

Sample collection for microbial diversity and population

On the fifteen day, chloroform anesthetized animals were sacrificed, after which part of the gut (large intestine) was carefully excised and weighed. The samples were then placed into different sterile universal bottles containing 9 ml of normal saline in an insulated ice-packed container as described by Chessbrough (2000).

Bacteria characterization and identification test

After collection of samples, the large intestine was homogenized with normal saline, 1 g was weighed, and serial dilutions were made to the sixth factor. The third and the fifth dilutions were introduced onto MacConkey and nutrient agar and sub–cultured in nutrient agar after 24 h to determine the bacteria count. Various biochemical tests (gram stain, oxidase, catalase, citrate, motility, indole and triple sugar ion) were carried out to determine the identity and as such, diversity of bacteria isolates (Cheesbrough, 2000)

Statistical analysis

The data obtained were presented as mean ± standard deviation (SD), subjected to analysis of variance (ANOVA) for differences between groups, using statistical package for social sciences (SPSS) software 23.0. A level of $p<0.05$ was considered as statistically significant for all tests.

RESULTS

The biochemical test carried out to identify the various bacterial isolates and also the cultural morphology is presented in Table1. The bacteria isolated include: *Salmonella* species, *Escherichia coli*, *Proteus* species, *Klebsiella* species, *Shigella* species, *Bacillus* species, *Morganella morgani*, *Lactobacillus* species, *Streptococcus* species, *Staphylococcus* species, *Bifidobacterium* species, *Pseudomonas* species *and Micrococcus* species.

There was a statistically significant increase in the bacteria count of animals administered caffeine (20 mg/kg), nicotine from cigarette (170 mg/kg), nicotine from cigarette (340 mg/kg), nicotine from tobacco (170 mg/kg), caffeine (20 mg/kg) and nicotine from cigarette (340mg/kg), caffeine (10 mg/kg) and nicotine from tobacco (170 mg/kg) as compared with the control group. Although the rest of the groups showed differences in the bacteria count when compared with the control, they were not statistically significant (Table 2).

The study revealed the presence of *E.coli*, *Shigella* species, *Bacillus* species, *M. morganii*, *L.* species, *Streptococcus* species, and *Staphylococcus* species in the control group. *Bacillus* species was present in all the groups except the group administered caffeine 10 mg/kg and nicotine from cigarette 170 mg/kg. *M. morganii* that was present in the control group was absent in the rest of the groups except the group administered nicotine from tobacco 340mg/kg and groups administered caffeine 20mg/kg, and nicotine from tobacco 340 mg/kg. *Staphylococcus* species however was present in the control group but was absent in the groups

Table1. Biochemical and cultural characteristics of bacterial isolates of animals administered caffeine and nicotine.

Cultural and morphological characteristics	Catalase	Oxidase	Indole	Glucose	Lactose	H_2S	Acid	Gas	Citrate	Motility	Gram stain	Probable organism
Cream, ovoid and discrete shaped cells	+	-	+	-	+	+	+	+	+	-	-	*Salmonella* sp
Cream colonies entire margin	+	-	+	+	+	+	+	+	-	+	-	*Escherichia coli*
Large yellowish cream colonies with undulated margin	+	-	-	+	+	+	+	+	-	+	-	*Proteus* sp
Tiny cream colonies rod	+	-	-	+	+	-	-	-	+	-	-	*Klebsiella pneumoniae*
Cream ovoid and discrete	+	-	-	+	+	-	+	+	+	-	-	*Shigella* sp
Cream ovoid colonies	+	+	+	+	+	+	+	-	+	-	+	*Bacillus* sp
Pink ovoid colonies and discrete	+	-	+	+	-	-	+	+	-	+	-	*Morganella morganii*
Cream ovoid colonies	-	-	+	+	+	-	+	-	-	+	+	*Lactobacillus* sp
Pink ovoid colonies	-	-	-	+	+	-	-	-	-	+	+	*Streptococcus* sp
Pink ovoid colonies	+	-	-	+	+	-	+	+	+	-	+	*Staphylococcus* sp
Pink ovoid colonies	-	-	-	+	+	-	-	-	+	+	+	*Bifidobacterium* sp
Cream ovoid colonies	+	+	-	+	-	-	-	-	+	+	-	*Pseudomonas* sp
Soft smooth yellowish growth	-	-	+	+	+	-	+	+	+	-	+	*Micrococcus* sp

(-)= Negative; (+)= Positive.

Table 2. Quantification of microorganisms in the gut of rats administered caffeine and nicotine.

Groups	Bacteria Count (cfu/g)
Control group 1	75.00±2.89
Group 2	82.500±6.29
Group 3	200.00±57.74*
Group 4	325.00±14.43*
Group 5	250.00±20.41*
Group 6	375.00±14.43*
Group 7	30.00±0.58
Group 8	405.00±36.86*
Group 9	412.00±31.46*
Group 10	375.00±43.30*
Group 11	150.00±84.16

*The mean difference is statistically significant compared to the control ($p < 0.05$).

KEY: Group 1 (served as control); Group 2 (Caffeine 10mg/kg); Group 3 (caffeine 20mg/kg); Group 4 (nicotine from cigarette 170mg/kg); Group 5 (nicotine from cigarette 340mg/kg); Group 6 (nicotine from tobacco 170mg/kg); Group 7 (nicotine from tobacco 340mg/kg); Group 8 (Caffeine 10mg/kg and nicotine from cigarette 170mg/kg); Group 9 (Caffeine 20mg/kg and nicotine from cigarette 340mg/kg); Group 10 (Caffeine 10mg/kg and nicotine from tobacco 170mg/kg); Group 11 (Caffeine 20mg/kg and nicotine from tobacco 340mg/kg).

administered caffeine 10 mg/kg and nicotine from 170 mg/kg and the groups administered caffeine 20 mg/kg and nicotine from tobacco 340 mg/kg while *Pseudomonas* species was only seen in the group administered commercially purchased caffeine 20 mg/kg as presented in Table 3.

DISCUSSION

The observed significant increase in the gut bacteria population of Wistar rats administered caffeine 20mg/kg (200.00±57.74) in Table 2 may be as a result of the ability of the organism to utilize caffeine for energy and growth, this is in line with the findings of Dash and Gummadi (2006) who opined that *Pseudomonas* species and some other bacteria utilize caffeine to improve and maintain growth. It was observed that there was a significant increase in the bacteria population of Wistar rats gut administered the combination of caffeine 20 mg/kg and nicotine from cigarette 340 mg/kg (412.00±31.46). The reason for this increase is not very clear but

Table 3. Diversity of bacteria isolated from animals administered caffeine and nicotine.

Groups/organism	A	B	C	D	E	F	G	H	I	J	K	L	M
Control group 1	-	++	-	-	++	+++++	+	+	+	++	-	-	-
Group 2	+	-	++	+	++	+	-	-	-	+	+	-	-
Group 3	++	-	-	-	-	+	-	+	+	++++	+	++	-
Group 4	-	++	-	+	+++	+	-	-	-	+++	-	-	-
Group 5	-	++++	-	++	-	++	-	-	+	+++	-	-	-
Group 6	-	-	++	-	-	+	++	-	-	+++	+	-	-
Group 7	-	-	-	-	-	++	-	+	++++	++	-	-	+
Group 8	++	-	-	+	+	-	-	+	++	-	-	-	-
Group 9	+	+++	-	++	+	++	-	-	+	++++	-	-	-
Group 10	+++	-	++	-	-	++	-	++	-	+	++	-	+
Group 11	-	-	+	+++	-	++	+	++++	+++	-	-	-	+

Key: A=*Samonella species,* B= *Escherichia coli,* C= *Proteus species,* D= *Klebsiella species,* E= *Shigella species,* F= *Bacillus species,* G= *Morganella morganii,* H= *Lactobacillus species,* I= *Streptococcus specie, J= Staphylococcus species, K= Bifidobacterium species,* L= *Pseudomonas species, M= Micrococcus species.*
Key: Group 1 (served as control), group 2 (Caffeine 10mg/kg), group3 (caffeine 20mg/kg), group 4 (nicotine from cigarette 170mg/kg), group 5 (nicotine from cigarette 340mg/kg), group 6 (nicotine from tobacco 170mg/kg), group 7 (nicotine from tobacco 340mg/kg), group 8 (Caffeine 10mg/kg and nicotine from cigarette 170mg/kg), group 9 (Caffeine 20mg/kg and nicotine from cigarette 340mg/kg), group 10 (Caffeine 10mg/kg and nicotine from tobacco 170mg/kg), group 11 (Caffeine 20mg/kg and nicotine from tobacco 340mg/kg).
(-) = Absent (+) = Present/degree of abundance.

it might be due to the co- administration of caffeine and nicotine. The co-administration may have enhanced the population of bacteria in the large intestine. This finding is consistent with the work of Biedermann et al. (2013).

Micrococcus species has the least prevalence across the groups while *Staphylococcus* species and *Bacillus* species appear to be the most prevalent of the bacteria isolated, being present in most groups. This study goes to show that the microbiota of human gut may change as a result of some food, drugs, age, or disease. This is in accordance with the findings of Rakaoff-Nahoum et al. (2016) who stated that the gut microbiota in humans changes and evolves throughout life, playing a significant role in both physiological and diseased conditions; it also changes when diet and overall health changes. However, there is paucity of information on this novel line of research.

There was little or no decrease in the diversity of *Staphylococcus* species across the groups despite the fact that they were administered with different agents. This may suggest that population and diversity is fairly stable in spite of the agents administered. There was a decrease in the prevalence of *Bacillus* species when compared to the control, this may suggest that a little alteration in meals, administration of drugs and other conditions may easily affect the abundance of *Bacillus* species in the gut (large intestine).

In all, these agents altered the distribution of bacteria species in the gut and this may result in a state of opportunistic infection. Macfarlane and Macfarlane (2003) suggested that altering the population and diversity of the gut flora can cause a reduction in the body's ability to ferment carbohydrate and break down bile acids, and this may cause redistribution. Carbohydrate that are not digested may absorb too much water and cause watery stools or lack of short chain fatty acids (SCFA) produced by the microbiota in the gut could cause diarrhea. An increase or decrease in the bacteria in the microbiota in humans has been implicated in several diseases such as inflammatory bowel disease, irritable bowel syndrome and a host of others (Kinross et al., 2011).

This study has shown that nicotine and caffeine utilization can alter the diversity and increase the population of microbes in the gut. This has a huge implication on the health of consumers of these agents. It is therefore important to carry out further studies to ascertain the pathogenicity of the isolated bacterial population. Further study is also required to isolate and characterize the fungal microbial population in the gut as a result of administration of the agents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adebayo, J.O., Akinyinka, A.O., Odewole, G. A., and Okwusichi J.I. (2007). Effect of caffeine on the risk of coronary heart disease-A re-evaluation. *Indian Journal of Clinical Biochemistry,* **22**(1): 29-32.

Armstrong, L.E., Casa, D.J., Maresh, C.M., and Ganio, M. (2007). Caffeine, fluid-electrolyte balance, temperature regulation, and exercise-heat tolerance. *Exercise and Sport Science Reviews* **35**(3): 135-140.

Biala, G., and Weglinska, B. (2004). Calcium channel antagonists attenuate cross-sectional sensitization to the rewarding and / or locomotors effect of nicotine, morphine. J. Pharm. Pharmacol., **56**(8) 1021-1028.

Biedermann, L., Jonas, Z., Jessica, M., Eveline, S., Ateequr, R., Stephan J., Claudia S., Anja, F., Pascal F., Michael S., Martin, J., Loessner Stephan, R., Michael, F., Stefan S., Markus S., and Gerhard, R. (2013). Smoking Cessation Induces Profound Changes in the Composition of the Intestinal Microbiota in Humans. *Plos One*, **8**(3): e59260 .

Cheesbrough, M. (2000). *District Laboratory practice in tropical countries*. Cambridge low price edition. Cambridge universal press.

Dash, S.S., and Gummadi, S.N. (2008). Inhibitory Effect of Caffeine on Growth of Various Bacterial Strains. Res. J. Microbiol., **3**: 457-465.

Fisone, G., Borgkvist, A., and Usiello, A. (2004). Caffeine as a psychomotor stimulant: mechanism of action. *Cellular and Molecular Life Sciences*. **61**(7–8): 857–72.

Garret, B.E., Rose, C.A., and Hennigfield, J.E. (2001). Tobacco addiction and pharmacological interventions. *Expert Opinion Pharmacotherapy*, **2**(10): 1545–1555.

Jaquet, M., Rochat, I., Moulin, J., Cavin, C., and Bibilon, R. (2009). Impact of coffee consumption on the gut microbiota: A human volunteer study. Inter. J. Food Microbiol., **130**(2): 117-121

Karch, S.B. (2009). *Karch's pathology of drug abuse* (4th edition). BocaRaton: CRC Press. pp. 229–230. ISBN 9780849378812.

Karina, C., Montan, M.F., Bergamaschi, C.C., Andrade, E., Rosalen, P.L., and Groppo, F.C. (2008). In vitro evaluation of the effect of nicotine, cotinine, and caffeine on oral microorganisms. *Can.* J. Microbiol., **54**(6):501-508.

Kinross, J.M., Ara, W.D. and Jeremy, K.N. (2011). Gut microbiome-host interactions in health and disease. *Genome Medicine*, **3**:14

Macfarlane, S., and Macfarlane, G.T. (2003). Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society*. **62**(1): 67–72.

Malenka, R.C., Nestler, E.J. and Hyman, S.E. (2009). Reinforcement and Addictive Disorders. *A Foundation for Clinical Neuroscience (2nd edition*.).New York: McGraw-Hill Medical. p. 375.ISBN 9780071481274.

Pavia, D.L., Lampman, G.M. and Kriz, G. S. Jr. (1976). *Introduction to organic laboratory technique*, W. B. Saunders Co., Philadelphia, pp. 50-54.

Quigley, E.M. (2013). Gut bacteria in health and disease. *Gastroenterology Hepatology* (N Y), 9: 560–569

Ted, W., and Norman, J.T. (2004). Beverages Nutrition and Health. *Humana Press*, p.172. ISBN 1588291731.

Viano, H., Weiderpass, E., and Kleihues, P. (2001). Smoking cessation in cancer prevention. *Toxicology*, **166(**1-2): 47-52.