

EFFECTS OF INTOXICATING SUBSTANCES ON GUT AND ORAL CAVITY

Koyel Mondal¹, Priyanka Pal^{2*}

¹Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Kolkata- 700121

²Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Kolkata- 700121

*Corresponding Author at: Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Malir Math, Kolkata, Bara Kanthalia, West Bengal 700121

*Corresponding Email: priyankarpal97@gmail.com

ABSTRACT

The Human body harbors trillions of microorganisms, known as natural microflora or commensal microbes. They present all over body, forming complex ecosystem. Dysbiosis condition among themselves create unhealthy condition as all of them have significant function to play. However, the consumption of intoxicating substances such as betel nut, gutka, cigarette, alcohol can disrupt their balance of the microbiome, creating favorable condition to grow the opportunistic microbes and pathogen and leading to various diseases. This review aims to summarize the effect on natural microflora of the most exposed part of the body to these intoxicants particularly oral cavity and gut, their mode of action of causing diseases and altering normal microflora. We highlighted the risk of diseases, those are directly linked by having these intoxicants such oral and gastrointestinal cancer, cardiovascular diseases and other disorders. The relationship between intoxicating substances and the human microbiome provided valuable insights on prevention and treatment of related diseases.

Keywords: *Gut, intoxicating substances, microflora, oral cavity*

Introduction

Human body is the home to trillions of microorganisms including bacteria, fungi and viruses. Among them bacteria are more abundant. These microorganisms form normal flora of the body, providing nutrients and stable environment to host and form symbiotic relationship with them. They also play different roles in our body. Balance between the commensal microbes and the host is essential for the normal function of the body. An increase or decrease in their number can cause disturbance in body. Human mouth and gut are more frequently exposed with microbes through foods, air, water. Consuming of Intoxicating Substances like Betel nut, Gutka, Cigarette, alcohol causes disbalance between in commensal microbes of gut and oral cavity, facilitating the growth of opportunistic microbes and pathogens and ultimately leading to various diseases. (Reyaz Ahmad Khan, et al., 2023). Betel nut or areca nut is a seed of areca palm, composed of tannins, arecoline arecaidine, free fatty acids, polysaccharides etc. For preparing betel quid or betel paan betel nut is main ingredients. A toxic mixture of areca nut, tobacco, slaked lime, catechu is known as gutka, also define as smokeless tobacco. it is placed between cheek and gum for chewing and Often get with various flavors to enhances the ability of mouth fresheners (Bharat Sankhla, et al., 2018). When fined cut tobacco rolled in thin paper with different flavors or without flavors known as cigarette. Composed of approximately 600 ingredients, among of them at least 69 are carcinogen. Nicotine, tar, tobacco, arsenic, benzene, acrolein, carbon monoxide, hydrogen cyanide these are the few components of the cigarette those lead to make it carcinogenic (Helen Keane, et al., 2020; Yoon-Seok Seo, et al., 2023). Alcohol is another intoxicant, composed with only carbon, hydrogen and oxygen. Accumulation of alcohol metabolites lead to cause cancer like diseases (Alessia Stornetta, et al., 2017). All of these intoxicants ultimately cause cancer by disrupting the normal function of the body's normal microbiome. On the basis of environmental factors, personal's food habit, hygiene human oral microbiome can be two type core or pioneer microbes and variable microbes. Bacteria can colonize in the hard tissue of teeth or soft tissue

of oral mucosa of oral cavity. Microbes get access of nutrients from teeth, tongue, gums, cheeks, tonsils, gingival sulcus etc. Recent study showed that newborns acquire microflora during delivery, feeding and while exposing environmental factors. *Fusobacterium nucleatum* was the most common phyla found in the oral cavity, besides that more 11 phyla (Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Spirochaetes, Synergistetes, Saccharibacteria (TM7) and Gracilibacteria) were observed. Among them 54% are officially named, 14% are unnamed but cultivated and 32% are known but uncultivated. All of these phyla have distinct characteristic so all of them contain with specific microorganism. After birth *Streptococcus salivarius*, *Lactobacillus*, *Actinomyces*, and *Veillonella* like aerobic bacteria mainly can grow as the pioneer microorganism in the oral cavity (Priya Nimish Deo, et al., 2019). In the gut ecosystem, 10- 100 trillions of microbes were developed after birth and influenced by diet and host genotype. Factors like age, diet, antibiotics and consumption of intoxicating substances can affect the microbial stability of the gut. *Helicobacter pylori*, a spiral shaped member of gram negative Epsilonproteobacteria inhabits in the acidic pH of mucus layer of the stomach. Whose presence make drastic changes in the stomach microbiota. *H. pylori* has the positive and negative correlation with Spirochaetae and Bacteroides, Chloroflexi, and other phyla respectively (Gabriele Capurso, et al., 2017). Acid resistance bacteria like *Lactobacillus* sp, *Veillonella* sp and *Clostridium* sp are dominants in stomach microbiota. Whereas, species like *Escherichia coli*, *peptococcus* sp, *Klebsiella* sp, *Bacteriodes* sp show decline growth in acidic environment. *Corynebacterium* sp, *Proteus* sp, *Enterobacter* sp, *Propionibacterium* sp were found as constant bacteria in average concentration (Bruno Zilberstein, et al., 2007). N-nitroso compounds (NOCs) are the key component of the cigarette smoke and gutka. Which are formed by the reaction of nitrate or nitrite with any amino group of the body. NOCs are Predominantly found in meat, smoked food, salted fish. The main sources of nitrate and nitrite are vegetables like beetroot, spinach, and cabbages, drinking water (in most countries) and animal food like processed and smoked foods respectively. These N-nitroso

compound (N-nitrosodimethylamine) acts as alkylating agent, react with the DNA and cause mutation (Monireh Sadat Seyyedsalehi et al., 2023). Besides NOCs, complex mixture of nicotine, aldehyde, acrolein, heavy metals are found in the several intoxicating substances including cigarette smoke and ultimately cause dysbiosis. Restricted the crucial functions include intestinal development, barrier integrity, metabolism, immunity, inflammation and neurological signalling regulation performed by gut microbiome. Leading to cause diseases like irritable bowel syndrome, cardiovascular disease, obesity, rheumatoid arthritis, systemic lupus erythematosus, central nervous system disease and ultimately cancer (Xiaohua Gui et al., 2021). Not only in the gut, oral microbe like Firmicutes, Actinobacteria, Bacteroidetes also get effected and get disrupted their normal function such as, food digestion, maintenance of oral pH, immune system modulation, defensive mechanism etc. Some pathogenic microbes such as; *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida* species, *Entamoeba gingivalis* and *Porphyromonas gingivalis* may be present in the oral cavity due to poor oral hygiene. These microbes can cause diseases and disorders such as; gingivitis, pharyngitis, stomatitis, tonsillitis, sinusitis, dental decay, oral thrush, halitosis, periodontitis, oral cancer and so on (Ofonime M. Ogba, et al., 2017; Sandhya Singh Kushwaha, et al., 2021). Disbalanced microflora in gut or oral cavity is the main reason of getting susceptible to various diseases caused by intoxicants. Change the concentration of inhabits microbes facilitate the opportunistic organisms and pathogens to grow. Once their concentrations get increased, cause distress. Several studies showed direct correlation with the microbes and the disease like increased concentration of *Helicobacter pylori* causes gastric cancer, *Fusobacterium nucleatum* and *Porphyromonas gingivalis* cause oral cancer. Factors like life style, geographical status, genetical history may affect the ecosystem of gut and oral cavity. Maintaining oral hygiene, having proper diet, or taking probiotics like *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bifidobacterium bifidum* can restore the microflora by stimulating immune system, inducing apoptosis, reducing cancer related

symptoms (Stanislav Sitkin, et al.,2022).

Oral Microflora

Microbes are present every part of the body, affect every aspect of human life. Oral cavity, one of the densely populates sites of the human body, often referred as the entrance or gate of the gut or digestive tract. From the oral cavity foods and liquid reach to the stomach through esophagus. Variety of sites are available where microbes can colonize and each site have specific environment that allow specific microbes to grow, not all the microbes can grow all the parts of oral cavity (Neetu Sharma, et al.,2018; Nobuhiro Takahashi, et al.,2025; M Killan, et al.,2016).Phylum Firmicutes (gram positives with low GC content) such as species of Streptococcus, Gemella, Eubacterium, Selenomonas, Veillonella, and related ones, the Actinobacteria (gram positives with high GC content) such as species of Actinomyces, Atopobium, Rothia, and related ones, the Proteobacteria (gram negative) species of Neisseria, Eikenella, Campylobacter, and related ones, the Bacteroidetes (Gram negative bacteria) species of Porphyromonas, Prevotella, Capnocytophaga, and related ones, the Fusobacteria (obligate anaerobe, nonsporforming, gram negative species of Fusobacterium and Leptotrichia, and the TM7 phylum are more abundant in the oral cavity(Jørn A. Aas, et al.,2005). TheSubgingival dental plaque, Supragingival dental plaque, Gums, Cheek, Tonsil,Throat, Saliva, Tongue soft tissues oforal cavity are populated by various microbes, providing different growth environment(Lu Gao, et al., 2018). In the Supragingival sites saccharolytic microorganism like Streptococcus sp, Actinomyces sp are predominant, take nutrients from continuously supplied saliva, fermenting carbohydrates produce lactic acid and creating acidic environment. The subgingival sites contain gingival crevicular fluid(GCF) which derived from the blood plasma so nutritionally rich in amino acids and proteins. Asaccharolytic bacteria such Fusobacteria sp, Prevotella sp metabolize nitrogenous compound and creating neutral pH.Haemophilus sp abundant in cheeks. Sulphur compounds are being producing by asaccharolytic microorganisms in the tongue coating, causing oral malodour. Additionally oral

microflora also can form biofilm where 500 microbial species communicate with each other's, act cooperatively and form community (Nobuhiro Takahashi, et al.,2025; Lu Gao, et al.,2018). Principle bacterial genera found in the healthy oral cavity are –

- Gram positive(cocci)–*Abiotrophiasp.*,*Peptostreptococcus sp.*, *Streptococcus sp.*, *Stomatococcus sp.*
- Gram positive(rods)- *Actinomyces sp.*, *Bifidobacterium sp.*, *Corynebacterium sp.*, *Eubacterium sp.*, *Lactobacillus sp.*, *Propionibacterium sp.*, *Pseudoramibacter sp.*, *Rothia sp.*
- Gram negative(cocci) -*Moraxella*, *Neisseria*, *Veillonella*
- Gram negative(rods) - *campylobacter*, *Capnocytophaga*, *Desulfobacter*, *Desulfovibrio*, *Eikenella*, *Fusobacterium*, *Hemophilus*, *Leptotrichia*, *Prevotella*, *Selemonas*, *Simonsiella*, *Treponema*, *Wolinella*(Priya Nimish Deo et al.,2019).

Gut Microflora

Human intestine poses diverse community, where 500-1000 bacterial species, 1800 genera found. Its 150 times higher than the human genome so now it also known as a discrete “organ” within human body. Bacterial inoculation had developed after post-partum and stabilizes near adolescence and stay intact till adulthood. 90% human intestine are dominated by the Firmicutes, Bacteroidetes phyla and followed by Proteobacteria, Fusobacteria, Verrucomicrobia (JohnTsiaoussis,et al.,2019). Some factors effect the gut microbiome like, in vaginal delivery neonate are more exposed to the mother vaginal microbes and dominated by *Lactobacillus*, *Prevotella*, and *sneathia* genera. While in c-Section delivery newborn poses less diverse gut microbiome with more skin -Dwelling microbes like, *Staphylococcus*, *Corynebacterium*, *Propionibacterium* etc. Additionally, breast milk contains own bacteria such *Bifidobacterium sp.* *E. coli*, *Clostridium difficile*, *Bacteroides fragilis* and *lactobacilli*, *Enterobacteriaceae* spare dominant in formula fed infants(Zahraa Al Bander, et al.,2020;Elizabeth Thursby, et al.,2017). In Infants of rural Africa dominate by the *Actinobacteria*, *Bacteroidetes* phyla due to having starch,

fibre and plant polysaccharide in diet(Carlotta De Filippo, et al.,2010). *Helicobacter pylori* have a complex relationship with gut microbiome.It protects against allergic, auto immune disorders and inflammatory bowel disease. More over help to maintain balance and diversity of GI. Eradication of *H. pylori* altered the bacterial metabolism, associated with the presence of *Rhodococcus*, *Lactobacillus*, and *Sphingomonas* and changing the gastric microbiota by increasing proteobacteria, Enterobacteriaceae, Enterococcus sp and decreasing Actinobacteria sp(Elizabeth Thursby, et al.,2017). *Lactobacilli* spare the members of lactic acid bacteria play vital role in the gut eco system, Produce lactic acid as the main product of the carbohydrate metabolism. There are 17 species of *Lactobacillus* associated with the human gut. Not only the gut, also abundant in fermented food, plants etc. Used as probiotic for their potential health benefits (Jens Walter, et al.,2008).

Intoxicating Substances and their mode of action

A mixture of powdered tobacco, Betel nut or areca nut and slaked lime are formed a form of smokeless tobacco, gutka. Other component of gutka include perfumery compounds such as sandalwood and musk ketones and commercially available in tins and sachets. consumption of gutka is increasing day by day due to lack of awareness of its negative impact on health, easy access and less expensive. It is prior placed between teeth chewed then held against buccal mucosa for long duration chewed and sucked. Arcea nut also can be used as Betel quid or paan, a blend of areca nut, slaked lime, artificial sweeteners and sometimes tobacco wrapped in *Piper betel* leaf. Zarda and Khaini are others form of smokeless tobacco(Bharat Sankhla, et al.,2018; Fawad Javed, et al.,2009). Habitable gutka use has been directly associated with the several oral mucosal disorders including oral submucosa fibrosis (ORS), oral cancer and periodontal disease. Investigation showed that patient with ORS (excess accumulation of collagen fibre in the oral mucosa) intolerance to spicy food, rigidity lips, tongue and palate and limited mouth opening and tongue movement ability. Fine grains of areca nut cause mechanical injury to oral tissue and fascinating the binding of ground

tobacco to the injured mucosa, leading to membrane damage and morphological changes. More over it increase the arterial blood pressure, heart rate. Arcea nut, tobacco and slaked lime, all of them have the different pathophysiological event that ultimately cause mucosal disorders. Acrolein is the one of the key alkaloids (basic nitrogen containing compounds) that can bind to the DNA by covalently, transferring the alkyl group to the DNA, or binding to major or minor group of specific DNA sequence led to cause breakage of DNA strand and DNA damage, arrest the cell cycle at G2/M stage, suppresses the p53. Indirectly by generating reactive oxidative species or inhibiting DNA repair enzyme (such as O6-methylguanine-DNA methyltransferase (MGMT) cause DNA damage (Fawad Javed, et al., 2009). Combination of Tobacco and areca nut with slaked lime in the gutka increase the concentration of ROS in saliva and produce superoxide anion and hydrogen peroxide, high ROS level cause direct tissue damage. Tobacco specific Nitrosamines present in the saliva of the chewers which converts procarcinogen to the carcinogen, metabolites of the tobacco specific Nitrosamines, that react with nucleophilic sites of on DNA bases causing damage. Polycyclic aromatic hydrocarbons (PAHs) is a tobacco mutagen that activated by the microsomal cytochrome P450, an enzyme that play a significant role in the metabolism of tobacco carcinogen (Bharat Sankhla, et al., 2018). Amphipathic nature of the ethanol makes it rapid absorbed through passive diffusion by stomach and liver. Hydrophilic properties of ethanol allow it to distribute throughout the body's water compartments by forming quick hydrogen bond with water. First metabolite of ethanol is acetaldehyde, a group 1 carcinogen to human, which is formed by alcohol dehydrogenase (ADH) in liver. Another two enzyme, those are involved in ethanol metabolism are aldehyde dehydrogenase (ALDH) and cytochrome P450. Aldehyde dehydrogenase form acetate from acetaldehyde, which denote as less harmful than acetaldehyde. Where as cytochrome P450 oxidizes ethanol to acetaldehyde. Specific mutation in the ALDH2 gene leads to deficient enzyme activity causing disbalance condition of ADH and ALDH result in accumulation of acetaldehyde. Acetaldehyde is an electrophilic molecule, which can react with the nucleophilic

centers of cellular constituents. By the imine formation between carbonyl carbon of acetaldehyde and amino group of DNA nucleotides acetaldehyde-DNA adduct is formed. N2-ethyldeoxyguanosine, one of the major DNA adduct and potent mutagen. Acetaldehyde can alter DNA methylation pattern, Histone modification, protein misfolding (Alessia Stornetta, et al.,2018; Mikko T. Nieminen, et al.,2018; Tingting Yan, et al.,2020). By binding to the mitochondrial electron transport chain (specifically complex I and III) acetaldehyde form superoxide anion (O_2^-) which further react with other molecule and form additional reactive oxygen species (H_2O_2 , OH^-). Additionally, ROS also can be formed as byproduct of the acetaldehyde metabolism. While metabolizing of ethanol by cytochrome P450 ROS generate (Masato Tamura, et al.,2013). ROS causes oxidative damage to protein, nucleic acid, lipid and by forming toxic lipid radicals ROS initiates lipid peroxidation (Hsu-Sheng Yu, et al.,2010). Alcohol consumption has been linked to increase risk of oral cavity cancer, oropharynx cancer, hypopharynx cancer, colon cancer, liver cancer, rectum cancer, female breast cancer (Alessia Stornetta, et al.,2018). Smoking is a significant risk factor for numerous diseases including cardiovascular diseases, respiratory conditions and various form of diseases. Cigarette smoke is a mixture 7000 chemicals including gases like carbon monoxide, carbon dioxide, nitrogen oxides, particular matter like tar, nicotine polycyclic aromatic hydrocarbons(PAHs), organic compound like acetaldehyde, acrolein, toluene, phenol, cresol, metal ions like As, Ba, Cu, Cs, Cd, Mn, Na, Zn Fe, K, Li etc. (Xiaohua Gui, et al.,2021). Nicotine is primarily absorbed by lung but also can be absorbed by skin, GI tract. It increases heart rates, blood pressure and generates ROS. It can easily pass blood brain barrier and influences function of nervous system. Nicotine binds to the nicotine acetylcholine receptors (nAChRs), located on the surface of the neurons, muscles cells and tissue. When nicotine binds to the receptors leading to an influx of positively charged ions such calcium, sodium into the cells. These cations further allowing calcium entry and release neurotransmitters including dopamine, acetylcholine. (Neal L Benowitz, et al.,2010; Liang Chi, et al.,2017). Heavy metals

of the cigarette could cause dysbiosis in intestinal ecosystem like lead and arsenic exposure increase the abundance of *Barnesiella* and *firmicutes* and decreases *firmicutes* and *Bacteroidetes* respectively. PAHs in the cigarette smoke cause DNA damage, mutation that led to activation of oncogenes and inactivation of tumor suppressor genes, by forming covalent bond with DNA bases it also can form DNA adduct these are metabolized by the cytochrome P 450 enzymes, which convert them into reactive oxygen species. More over cigarette smoke itself contain with radicals and ROS which further contribute to produce oxidative stress. It also can disrupt the electron transfer chain of mitochondria causing mitochondrial damage (Xiaohua Gui, et al.,2021., William A Pryor, et al., 1976).

Conclusion

In conclusion, dysbiosis or alteration of the functions of commensal microbes in any parts of body remarks great changes. Prolong Ignorance of changes cause harmful disease. All of the microbes have specific and significant role in our body. All of the intoxicating has some similar composition in it like aldehyde, nicotine, heavy metals etc. Their ultimate mode of action is similar in the body. Generation of free radicals or reactive oxidative species is main cause of various disease by forming DNA adduct or mutation. Genetic evolution or genetic history also play a crucial role of having disease in offspring. Future investigation is going on long term effect of intoxicant use on physical and mental health, effect of geographical differences, effect of e- cigarette and vaping product use, role of specific microflora preventing harmful effect of intoxicants. To avoid the addiction of intoxicating conveying the awareness about the risks and consequences of intoxicant use can be effective way. By organising health fairs, public service announcements, public lectures, workshops or providing counselling to the addicted people or establishing supportive group awareness can be spreaded. Self-realising is very crucial to total get rid from the addiction.

References:

Khan RA, Devi KR, Pratim Barman M, Bhagawati M, Sarmah R. Bacteria in the oral cavity of individuals consuming intoxicating substances. *PLoS One*. 2023 May 26;18(5): e0285753. doi: 10.1371/journal.pone.0285753. PMID: 37235563; PMCID: PMC10218728.

Sankhla B, Kachhwaha K, Hussain SY, Saxena S, Sireesha SK, Bhargava A. Genotoxic and Carcinogenic Effect of Gutkha: A Fast-growing Smokeless Tobacco. *Addict Health*. 2018 Jan;10(1):52-63. doi: 10.22122/ahj. v10i1.537. PMID: 30627385; PMCID: PMC6312563.

Seo YS, Park JM, Kim JH, Lee MY. Cigarette Smoke-Induced Reactive Oxygen Species Formation: A Concise Review. *Antioxidants (Basel)*. 2023 Sep 7;12(9):1732. doi: 10.3390/antiox12091732. PMID: 37760035; PMCID: PMC10525535.

Stornetta A, Guidolin V, Balbo S. Correction: Alessia Stornetta et al. Alcohol-Derived Acetaldehyde Exposure in the Oral Cavity. *Cancers* 2018, 10, 20. *Cancers (Basel)*. 2018 Mar 16;10(3):74. doi: 10.3390/cancers10030074. Erratum for: *Cancers (Basel)*. 2018 Jan 14;10(1): E20. doi: 10.3390/cancers10010020. PMID: 29547517; PMCID: PMC5876649.

Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. *J Oral Maxillofac Pathol*. 2019 Jan-Apr;23(1):122-128. doi: 10.4103/jomfp.JOMFP_304_18. PMID: 31110428; PMCID: PMC6503789.

Capurso G, Lahner E. The interaction between smoking, alcohol and the gut microbiome. *Best Pract Res Clin Gastroenterol*. 2017 Oct;31(5):579-588. doi: 10.1016/j.bpg.2017.10.006. Epub 2017 Oct 22. PMID: 29195678.

Zilberstein B, Quintanilha AG, Santos MA, Pajacki D, Moura EG, Alves PR, Maluf Filho F, de Souza JA, Gama-Rodrigues J. Digestive tract microbiota in healthy volunteers. *Clinics (Sao Paulo)*. 2007 Feb;62(1):47-54. doi: 10.1590/s1807-59322007000100008. Erratum in: *Clinics*. 2007 Apr;62(2):206. PMID: 17334549.

Seyyedsalehi MS, Mohebbi E, Tourang F, Sasanfar B, Boffetta P, Zendehdel K. Association of Dietary Nitrate, Nitrite, and N-Nitroso Compounds Intake and Gastrointestinal Cancers: A Systematic Review and Meta-Analysis. *Toxics*. 2023

Feb 17;11(2):190. doi: 10.3390/toxics11020190. PMID: 36851064; PMCID: PMC9962651.

Gui X, Yang Z, Li MD. Effect of Cigarette Smoke on Gut Microbiota: State of Knowledge. *Front Physiol.* 2021 Jun 17; 12:673341. doi: 10.3389/fphys.2021.673341. PMID: 34220536; PMCID: PMC8245763.

Kushwaha SS, Multani RK, Kushwaha NS, Gautam S, Jindal DG, Arora KS, Avasthi A. Saliva as a Potential Diagnostic Tool to Evaluate Relationship between Oral Microbiome and Potentially Malignant Disorders for Prevention of Malignant Transformation. *Asian Pac J Cancer Prev.* 2021 Jan 1;22(1):125-129. doi: 10.31557/APJCP.2021.22.1.125. PMID: 33507689; PMCID: PMC8184171.

Sitkin S, Lazebnik L, Avalueva E, Kononova S, Vakhitov T. Gastrointestinal microbiome and *Helicobacter pylori*: Eradicate, leave it as it is, or take a personalized benefit-risk approach? *World J Gastroenterol.* 2022 Feb 21;28(7):766-774. doi: 10.3748/wjg.v28.i7.766. PMID: 35317277; PMCID: PMC8891730.

Sharma N, Bhatia S, Sodhi AS, Batra N. Oral microbiome and health. *AIMS Microbiol.* 2018 Jan 12;4(1):42-66. doi: 10.3934/microbiol.2018.1.42. PMID: 31294203; PMCID: PMC6605021.

Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol.* 2005 Nov;43(11):5721-32. doi: 10.1128/JCM.43.11.5721-5732.2005. PMID: 16272510; PMCID: PMC1287824.

Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: more and more importance in oral cavity and whole body. *Protein Cell.* 2018 May;9(5):488-500. doi: 10.1007/s13238-018-0548-1. Epub 2018 May 7. PMID: 29736705; PMCID: PMC5960472.

Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, Tonetti MS, Wade WG, Zaur E. The oral microbiome - an update for oral healthcare professionals. *Br Dent J.* 2016 Nov 18;221(10):657-666. doi: 10.1038/sj.bdj.2016.865. PMID: 27857087.

Tsiaoussis J, Antoniou MN, Koliarakis I, Mesnage R, Vardavas CI, Izotov BN, Psaroulaki A, Tsatsakis A. Effects of single and combined toxic exposures on the gut microbiome: Current knowledge and future directions. *Toxicol Lett.* 2019 Sep 15; 312:72-97. doi: 10.1016/j.toxlet.2019.04.014. Epub 2019

Apr 27. PMID: 31034867.

Al Bander Z, Nitert MD, Mousa A, Naderpoor N. The Gut Microbiota and Inflammation: An Overview. *Int J Environ Res Public Health*. 2020 Oct 19;17(20):7618. doi: 10.3390/ijerph17207618. PMID: 33086688; PMCID: PMC7589951.

Thursby E, Juge N. Introduction to the human gut microbiota. *Biochem J*. 2017 May 16;474(11):1823-1836. doi: 10.1042/BCJ20160510. PMID: 28512250; PMCID: PMC5433529.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A*. 2010 Aug 17;107(33):14691-6. doi: 10.1073/pnas.1005963107. Epub 2010 Aug 2. PMID: 20679230; PMCID: PMC2930426.

Walter J. Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Appl Environ Microbiol*. 2008 Aug;74(16):4985-96. doi: 10.1128/AEM.00753-08. Epub 2008 Jun 6. PMID: 18539818; PMCID: PMC2519286.

Javed F, Chotai M, Mehmood A, Almas K. Oral mucosal disorders associated with habitual gutka usage: a review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010 Jun;109(6):857-64. doi: 10.1016/j.tripleo.2009.12.038. Epub 2010 Apr 9. PMID: 20382045.

Nieminen MT, Salaspuro M. Local Acetaldehyde-An Essential Role in Alcohol-Related Upper Gastrointestinal Tract Carcinogenesis. *Cancers (Basel)*. 2018 Jan 5;10(1):11. doi: 10.3390/cancers10010011. PMID: 29303995; PMCID: PMC5789361.

Yan T, Zhao Y. Acetaldehyde induces phosphorylation of dynamin-related protein 1 and mitochondrial dysfunction via elevating intracellular ROS and Ca²⁺ levels. *Redox Biol*. 2020 Jan; 28:101381. doi: 10.1016/j.redox.2019.101381. Epub 2019 Nov 11. PMID: 31756635; PMCID: PMC6879985.

Tamura M, Matsui H, Kaneko T, Hyodo I. Alcohol is an oxidative stressor for gastric epithelial cells: detection of superoxide in living cells. *J Clin Biochem Nutr*. 2013 Sep;53(2):75-80. doi: 10.3164/jcbn.13-32. Epub 2013 Aug 31. PMID: 24062603; PMCID: PMC3774929.

Yu HS, Oyama T, Isse T, Kitagawa K, Pham TT, Tanaka M, Kawamoto T. Formation of acetaldehyde-derived DNA adducts due to alcohol exposure. *Chem Biol Interact.* 2010 Dec 5;188(3):367-75. doi: 10.1016/j.cbi.2010.08.005. Epub 2010 Sep 21. PMID: 20813101. Seo YS, Park JM, Kim JH, Lee MY. Cigarette Smoke-Induced Reactive Oxygen Species Formation: A Concise Review. *Antioxidants (Basel).* 2023 Sep 7;12(9):1732. doi: 10.3390/antiox12091732. PMID: 37760035; PMCID: PMC10525535.

Benowitz NL. Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. *Annu Rev Pharmacol Toxicol.* 2009; 49:57-71. doi: 10.1146/annurev.pharmtox.48.113006.094742. PMID: 18834313; PMCID: PMC2946180.

Chi L, Mahbub R, Gao B, Bian X, Tu P, Ru H, Lu K. Nicotine Alters the Gut Microbiome and Metabolites of Gut-Brain Interactions in a Sex-Specific Manner. *Chem Res Toxicol.* 2017 Dec 18;30(12):2110-2119. doi: 10.1021/acs.chemrestox.7b00162. Epub 2017 Nov 16. PMID: 29035044.

Pryor WA. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. *Environ Health Perspect.* 1997 Jun;105 Suppl 4(Suppl 4):875-82. doi: 10.1289/ehp.97105s4875. PMID: 9255574; PMCID: PMC1470037.