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Acetaminophen Induced Liver Injury: Metabolism and Inflammation Perspectives

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Abstract: Acetaminophen (APAP) overdose is the most common cause of acute liver failure in the US, and decades of intense study of its pathogenesis resulted in the development of the antidote *N*-acetylcysteine, which facilitates scavenging of the reactive metabolite and is the only treatment in clinical use. However, the narrow therapeutic window of this intervention necessitates a better understanding of the intricacies of APAP-induced liver injury for the development of additional therapeutic approaches that can benefit late-presenting patients. More recent investigations into APAP hepatotoxicity have established the critical role of mitochondrial dysfunction in mediating liver injury as well as clarified mechanisms of APAP-induced hepatocyte cell death facilitates scavenging of the reactive metabolite and is the only treatment in clinical use. Mitochondrial oxidative and nitrosative stress is a key mechanistic feature involved in downstream signaling after APAP overdose. The identification of specific mediators of necrotic cell death further establishes the regulated nature of APAP-induced hepatocyte cell death. In addition, the discovery of the role of mitochondrial dynamics and autophagy in APAP-induced liver injury provides additional insight into the elaborate cell signaling mechanisms involved in the pathogenesis of this important clinical problem. In spite of these new insights into the mechanisms of liver injury, significant controversy still exists on the role of innate immunity in APAP-induced hepatotoxicity.

Keywords: Acetaminophen toxicity, Hepatotoxicity, Acute liver failure (ALF)

INTRODUCTION

Acetaminophen (APAP) is one of the most common analgesic and antipyretic drugs in use globally.(Lee, 2008) Though the drug is safe and effective at therapeutic doses.(Anitha and Ashwini, 2017) The therapeutic window is narrow, and an overdose is highly hepatotoxic.(Larson *et al.*, 2005) Because of the ubiquitous nature and broad availability of the drug has resulted in APAP induced hepatotoxicity being the most frequent cause of acute liver failure (ALF) in the US and other Western countries(Ashwini, Ezhilarasan and Anitha, 2017) (Larson *et al.*, 2005) Acetaminophen induced hepatotoxicity remains as a significant public health concern and common indication for emergent liver transplantation as the most common cause of ALF in the USA and UK.(Bateman *et al.*, 2014)(Lakshmi *et al.*, 2015) Acetaminophen combination products frequently prescribed by physicians and other healthcare professionals, with unintentional and chronic overdose accounting for over 50% of cases of APAP-related ALF.(Lancaster, Hiatt and Zarrinpar, 2015; Sharma *et al.*, 2019) APAP hepatotoxicity remains a global issue; it accounts for more than 50% of overdose-related acute liver failure in the united states and approximately 20% of the liver transplant cases.(Nelson, 1990; Yoon *et al.*, 2016) The pathophysiology of acute liver failure secondary to APAP toxicity remains to be precisely elucidated, and has adverse patient outcomes such as increased morbidity and mortality.(Ezhilarasan, Lakshmi, Vijayaragavan, *et al.*, 2017) (Xie *et al.*, 2015) Decades of investigations into the mechanisms of APAP-induced liver injury have provided significant insight into the role of APAP metabolism and formation of a reactive metabolite in initiating the cascade of events ultimately leading to liver injury.(Court *et al.*, 2017) Hepatic injury and subsequent hepatic failure due to both intentional and unintentional overdose of acetaminophen (APAP) has affected patients for decades, and involves the cornerstone metabolic pathways which take place in the microsomes within hepatocytes.(McGill and Jaeschke, 2013) Oxidative stress, inflammation, mitochondrial dysfunction and hepatocytes death have been attributed to APAP intoxication in the liver.(Lancaster, Hiatt and Zarrinpar, 2015) (Perumalsamy *et al.*, 2018).Our team has rich experience in research and we have collaborated with numerous authors over various topics in the past decade (Deogade, Gupta and Ariga, 2018; Ezhilarasan, 2018; Ezhilarasan, Sokal and Najimi, 2018; Jeevanandan and Govindaraju, 2018; J *et al.*, 2018; Menon *et al.*, 2018; Prabakar *et al.*, 2018;

Rajeshkumar, Kumar, *et al.*, 2018; Vishnu Prasad *et al.*, 2018; Wahab *et al.*, 2018; Dua *et al.*, 2019; Duraisamy *et al.*, 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Gheena and Ezhilarasan, 2019a; Malli Sureshbabu *et al.*, 2019; Mehta *et al.*, 2019; Panchal, Jeevanandan and Subramanian, 2019; Rajendran *et al.*, 2019; Rajeshkumar *et al.*, 2019; Ramakrishnan, Dhanalakshmi and Subramanian, 2019; Sharma *et al.*, 2019; Varghese, Ramesh and Veeraiyan, 2019; Gomathi *et al.*, 2020; Samuel, Acharya and Rao, 2020)
The aim of this study is to understand the acetaminophen-induced liver injury via its metabolism and pro-inflammatory potential.

Metabolism of Acetaminophen
Acetaminophen is extensively metabolized by the liver via three main hepatic pathways: glucuronidation, sulfation, and CYP450 2E1 oxidation. Approximately 90% of acetaminophen is conjugated to sulfated and glucuronidated metabolites that are renally eliminated**.**Acetaminophen metabolism occurs in the hepatocytes microsomes.(Mehta *et al.*, 2019) At therapeutic doses, APAP (90%) undergo glucuronic acid or sulfate conjugation and is excreted through the kidneys (Myers *et al.*, 1995)(Ezhilarasan, Lakshmi, Nagaich, *et al.*, 2017). A minor component is acted upon by cytochrome P450 enzymes such as Cyp2E1 and Cyp1A2 to form a reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (Matthews, 1997). Though highly reactive, NAPQI is rarely harmful after consumption of therapeutic doses because it is rapidly conjugated with abundant intracellular antioxidants such as glutathione stores in the hepatocytes and excreted through the bile.(Hadi *et al.*, 2013) (Ezhilarasan, 2018) However, this contrasts to the scenario after consumption of an overdose of APAP, where the sulfation pathway is saturated and NAPQI generation is significantly elevated in spite of the high capacity of the glucuronidation pathway.(Qiu, Benet and Burlingame, 1998) (McGill and Jaeschke, 2013)This results in robust reaction with hepatic glutathione stores and the subsequent rapid depletion of glutathione stores in the hepatocytes of the liver.(Andringa *et al.*, 2008) Metabolism of APAP can be influenced by genotype differences, and variations in glucuronidation are seen in different populations due to polymorphisms in the UDP-glucuronosyltransferase (UGT) enzymes.(Bruderer *et al.*, 2015). *N*-acetylcysteine is reported to facilitate scavenging of APAP derived reactive metabolites and is the only treatment for APAP overdose. (Wong *et al.*, 2020). The mechanism of APAP metabolism associated with changes in liver is presented in Figure 1.

Acetaminophen Induced Liver Damage
Hepatic injury and subsequent hepatic failure due to both intentional and unintentional overdose of acetaminophen (APAP) has affected patients for decades, and involves the cornerstone metabolic pathways which take place in the microsomes within hepatocytes.Acetaminophen-induced hepatotoxicity remains a significant public health concern and is a common indication for emergent liver transplantation (Jaeschke, 2003). (Moalem, 2020) APAP associated ALF cases have non-specific symptoms such as nausea, vomiting, and malaise.(Ezhilarasan, 2018; Gheena and Ezhilarasan, 2019b) Severe acute liver injury often leads to impaired elimination of bilirubin, manifesting as jaundice immediately prior to or shortly after presentation. In addition, the depressed synthesis and excessive consumption of clotting factors results in a complex coagulopathy.(McGill *et al.*, 2012) A diminished synthesis of glucose, increased intracellular lactate production, and the reduction in hepatic uptake of lactate leads to hypoglycemia, and metabolic acidosis.(Menon *et al.*, 2018) Thirty to forty percent of ALF patients present with impaired renal function and associated azotemia and oliguria.(Ring-Larsen and Palazzo, 1981). (Jaeschke, 2015)Liver damage from APAP can be severe, which can result either from an overdose or from regular doses that are taken while drinking alcohol.(Bruderer *et al.*, 2015) Most of APAP induced liver injury cases are caused by an intentional or suicidal overdose.(Cooper *et al.*, 2009) Oxidative stress mediated by hepatotoxic APAP metabolite N-acetyl-p-benzoquinoneimine (NAPQI), is considered as the main cause of hepatocellular degeneration. (Murray *et al.*, 2008). APAP causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose.(Court *et al.*, 2017). APAP protein adducts that form upon NAPQI conjugation with protein sulfhydryl groups of cysteine in GSH or cellular proteins readily interact with mitochondria.(Holt, Cheng and Ju, 2008) These mitochondrial protein adducts are thought to cause mitochondrial dysfunction and to promote oxidative stress.(Xie *et al.*, 2014),(Yang *et al.*, 2015) Therefore, detection of elevated levels of these APAP-protein adducts can be used for clinical indication of APAP hepatotoxicity.(Antoine *et al.*, 2015).
Other mechanisms of hepatotoxicity induced by APAP include the formation of toxic free radicals, such as peroxynitrite, from the reaction of superoxide and nitric oxide, subsequently forming nitrotyrosine adducts inside the mitochondria.(Jaeschke *et al.*, 2012). GSH repletion not only provides surplus cysteine as an energy substrate for the Krebs cycle, it also serves the important role of scavenging for free radicals and peroxynitrite(Menon *et al.*, 2018; Rajeshkumar, Kumar, *et al.*, 2018).Mitochondria, are critical for cellular respiration and the metabolism, and suffers damage to their own mitochondrial DNA by the actions of ROS and peroxynitrite compounds, and they have been directly implicated in the process leading to cessation of ATP synthesis.(Liu, Govindarajan and Kaplowitz, 2004) The detection of one or more biomarkers in the setting of APAP hepatotoxicity is desirable, especially in clinical scenarios, the diagnosis of APAP hepatotoxicity is

unclear. Multiple serum biomarkers are potential indicators, not only to identification of hepatocyte injury and necrosis but also to help predict the patient outcomes based on the presence or absence of certain intracellular or intra mitochondrial markers.(Dambach *et al.*, 2002) (Karthiga, Rajeshkumar and Annadurai, 2018) Serum markers of mitochondrial damage and death including glutamate dehydrogenase (GDH), nuclear DNA and mitochondrial DNA have been investigated as clinically useful surrogate markers capable of indicating mitochondrial lysis following hepatocyte necrosis in APAP hepatotoxicity(McGill, Cao, *et al.*, 2014; McGill, Li, *et al.*, 2014). The elevated GDH and alanine transaminase activity in plasma was found in patients with APAP induced liver injury (Thulin *et al.*, 2014), (Buckley, Eddleston and Szinicz, 2005).

Acetaminophen Induced Hepatic Inflammation

Acetaminophen overdose can result in serious liver injury and potentially death. APAP induced hepatotoxicity mainly depends on metabolism of APAP to a reactive metabolite initiating a cascade of intracellular events resulting in hepatocellular necrosis.(Leang *et al.*, 2014) (Rajeshkumar, Agarwal, *et al.*, 2018) An early APAP injury triggers sterile inflammatory response with formation of cytokines and innate immune cell infiltration in the liver.(Lee, 2004) The extensive hepatocellular necrosis after an APAP overdose leads to release of damage-associated molecular patterns (DAMPs) including mitochondrial DNA, nuclear DNA fragments, high-mobility group box (HMGB1) protein, and many others.(Hawton, 2001) DAMPs bind to pattern recognition receptors such as toll-like receptors (TLRs) on inflammatory cells and transcriptionally activate cytokine formation in inflammatory cells (Martin-Murphy, Holt and Ju, 2010). During APAP intoxication, inflammatory cells in the liver produce several proinflammatory markers which further aggravates liver inflammation. Diet restriction inhibits apoptosis and HMGB1 oxidation which promotes the inflammatory cell recruitment during acetaminophen hepatotoxicity. The role of damage is associated with the molecular pattern of molecules in acetaminophen-induced liver injury in mice.(Bunchorntavakul and Reddy, 2013) Due to some unique properties, IL-18 stands out among members of the IL-1 cytokine family.(Sandhu and Navarro, 2020) IL-18 is constitutively expressed in a variety of cell types, for example, in hepatic Kupffer cells Accordingly, IL-18 expression is detectable in healthy murine liver where macrophages/Kupffer cells are a major source of bioactive IL-18.(Mühl and Pfeilschifter, 2004),(Hayashi *et al.*, 2007)

The DAMPs release is required for the APAP-induced sterile inflammatory response. Previous experimental studies reported the release of DAMPs such as HMGB1, heat shock proteins, and DNA fragments in plasma after APAP administration in experimental animals (Sandhu and Navarro, 2020). During APAP induced-necrosis, the necrotic cells passively release hypo-acetylated HMGB1 and macrophages or monocytes actively secretes hyper-acetylated form of HMGB1. (James *et al.*, 2007) (Khandelwal *et al.*, 2011)Our institution is passionate about high quality evidence based research and has excelled in various fields ((Pc, Marimuthu and Devadoss, 2018; Ramesh *et al.*, 2018; Vijayashree Priyadharsini, Smiline Girija and Paramasivam, 2018; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Ramadurai *et al.*, 2019; Sridharan *et al.*, 2019; Vijayashree Priyadharsini, 2019; Chandrasekar *et al.*, 2020; Mathew *et al.*, 2020; R *et al.*, 2020; Samuel, 2021)

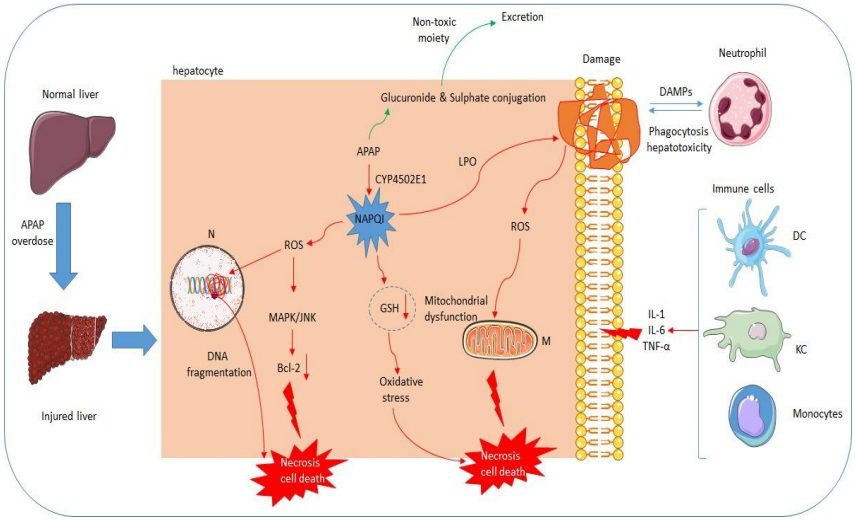

Fig.1: Mechanisms of acetaminophen (APAP) metabolism associated liver injury. LPO, lipid peroxidation; M, mitochondria; NAPQI, N-acetyl-p-benzoquinoneimine; GSH, reduced glutathione; IL, interlukin; TNF, tissue necrosis factor alpha; KC, Kupffer cells; DAMPs, damage-associated molecular patterns; ROS, reactive oxygen species; MAPK, mitogen activated protein kinase; JNK, c-Jun N-terminal kinase; N, nucleus

CONCLUSION

Over the last several decades, significant progress has been made in the understanding of the intracellular signaling mechanisms leading to APAP-induced cell death in hepatocytes in experimental animals and humans. Although more can be learned about various aspects of these mechanisms, it is important to keep in mind the potential effects of intervention strategies on drug metabolism, which can lead to misinterpretations. It is also critical to connect any newly discovered mediators and pathways to the established mechanisms. The recent studies have explored the role of the immune system, DAMPs and inflammasome in APAP-induced hepatotoxicity. Therefore, future experimental and clinical studies can target APAP- induced liver injury by employing novel anti inflammatory and immunomodulatory agents.

Source of Support
Nil

CONFLICT OF INTEREST

None Declared

Abbreviations

- APAP- Acetaminophen
- ALF-Acute liver failure
- DAMP-damage-associated molecular pattern
- GDCA-glycodeoxycholic acid
- GDH-glutamate dehydrogenase
- GSH-glutathione
- miRNA-microRNA
- mtDNA-mitochondrial DNA
- NAC-*N*-acetyl cysteine
- NAPQI-*N*-acetyl-para-benzo-quinone imine
- nDNA-nuclear DNA
- ROS- reactive oxygen species
- SULT-sulfotransferase
- UGT-UDP-glucuronosyltransferase

REFERENCE

1. Andringa, K. K. et al. (2008) 'Mitochondrial protein thiol modifications in acetaminophen hepatotoxicity: Effect on HMG-CoA synthase', Toxicology Letters, pp. 188–197. doi: 10.1016/j.toxlet.2008.01.010.
2. Anitha, R. and Ashwini, S. (2017) 'Antihyperglycemic activity of Caralluma fimbriata: An In vitro approach', Pharmacognosy Magazine, p. 499. doi: 10.4103/pm.pm_59_17.
3. Antoine, D. J. et al. (2015) 'Circulating Kidney Injury Molecule 1 Predicts Prognosis and Poor Outcome in Patients With Acetaminophen-Induced Liver Injury', Hepatology, pp. 591–599. doi: 10.1002/hep.27857.
4. Ashwini, S., Ezhilarasan, D. and Anitha, R. (2017) 'Cytotoxic Effect of Caralluma fimbriata Against Human Colon Cancer Cells', Pharmacognosy Journal, pp. 204–207. doi: 10.5530/pj.2017.2.34.
5. Bateman, D. N. et al. (2014) 'Effect of the UK's revised paracetamol poisoning management guidelines on admissions, adverse reactions and costs of treatment', British Journal of Clinical Pharmacology, pp. 610–618. doi: 10.1111/bcp.12362.
6. Bruderer, R. et al. (2015) 'Extending the Limits of Quantitative Proteome Profiling with Data-Independent Acquisition and Application to Acetaminophen-Treated Three-Dimensional Liver Microtissues', Molecular & Cellular Proteomics, pp. 1400–1410. doi: 10.1074/mcp.m114.044305.
7. Buckley, N., Eddleston, M. and Szinicz, L. (2005) 'Oximes for acute organophosphate pesticide poisoning', Cochrane Database of Systematic Reviews. doi: 10.1002/14651858.cd005085.
8. Bunchorntavakul, C. and Reddy, K. R. (2013) 'Acetaminophen-related hepatotoxicity', Clinics in liver disease, 17(4), pp. 587–607, viii.
9. Chandrasekar, R. et al. (2020) 'Development and validation of a formula for objective assessment of cervical vertebral bone age', Progress in orthodontics, 21(1), p. 38.
10. Cooper, S. C. et al. (2009) 'Outcomes of liver transplantation for paracetamol (acetaminophen)-induced hepatic failure', Liver Transplantation, pp. 1351–1357. doi: 10.1002/lt.21799.
11. Court, M. H. et al. (2017) 'Race, Gender, and Genetic Polymorphism Contribute to Variability in Acetaminophen Pharmacokinetics, Metabolism, and Protein-Adduct Concentrations in Healthy African-American and European-American Volunteers', Journal of Pharmacology and Experimental Therapeutics, pp. 431–440. doi: 10.1124/jpet.117.242107.
12. Dambach, D. M. et al. (2002) 'Role of CCR2 in macrophage migration into the liver during acetaminophen-

induced hepatotoxicity in the mouse', Hepatology , 35(5), pp. 1093–1103.
13. Deogade, S., Gupta, P. and Ariga, P. (2018) 'Effect of monopoly-coating agent on the surface roughness of a tissue conditioner subjected to cleansing and disinfection: A Contact Profilometric In vitro study', Contemporary Clinical Dentistry, p. 122. doi: 10.4103/ccd.ccd_112_18.
14. Dua, K. et al. (2019) 'The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress', Drug development research, 80(6), pp. 714–730.
15. Duraisamy, R. et al. (2019) 'Compatibility of Nonoriginal Abutments With Implants: Evaluation of Microgap at the Implant-Abutment Interface, With Original and Nonoriginal Abutments', Implant dentistry, 28(3), pp. 289–295.
16. Ezhilarasan, D., Lakshmi, T., Vijayaragavan, R., et al. (2017) 'Acacia catechu ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells', Journal of Advanced Pharmaceutical Technology & Research, p. 143. doi: 10.4103/japtr.japtr_73_17.
17. Ezhilarasan, D., Lakshmi, T., Nagaich, U., et al. (2017) 'Acacia catechu ethanolic seed extract triggers apoptosis of SCC-25 cells', Pharmacognosy Magazine, p. 405. doi: 10.4103/pm.pm_458_16.
18. Ezhilarasan, D. (2018) 'Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective', Arab journal of gastroenterology: the official publication of the Pan-Arab Association of Gastroenterology, 19(2), pp. 56–64.
19. Ezhilarasan, D., Apoorva, V. S. and Ashok Vardhan, N. (2019) 'Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells', Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology, 48(2), pp. 115–121.
20. Ezhilarasan, D., Sokal, E. and Najimi, M. (2018) 'Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets', Hepatobiliary & pancreatic diseases international: HBPD INT, 17(3), pp. 192–197.
21. Gheena, S. and Ezhilarasan, D. (2019a) 'Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells', Human & experimental toxicology, 38(6), pp. 694–702.
22. Gheena, S. and Ezhilarasan, D. (2019b) 'Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells', Human & Experimental Toxicology, pp. 694–702. doi: 10.1177/0960327119839173.
23. Gomathi, A. C. et al. (2020) 'Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line', Journal of Drug Delivery Science and Technology, p. 101376. doi: 10.1016/j.jddst.2019.101376.
24. Hadi, M. et al. (2013) 'AMAP, the alleged non-toxic isomer of acetaminophen, is toxic in rat and human liver', Archives of Toxicology, pp. 155–165. doi: 10.1007/s00204-012-0924-1.
25. Hawton, K. (2001) 'Effects of legislation restricting pack sizes of paracetamol and salicylate on self poisoning in the United Kingdom: before and after study', BMJ, pp. 1203–1203. doi: 10.1136/bmj.322.7296.1203.
26. Hayashi, N. et al. (2007) 'T helper 1 cells stimulated with ovalbumin and IL-18 induce airway hyperresponsiveness and lung fibrosis by IFN- and IL-13 production', Proceedings of the National Academy of Sciences, pp. 14765–14770. doi: 10.1073/pnas.0706378104.
27. Holt, M. P., Cheng, L. and Ju, C. (2008) 'Investigation of the Potential Anti-Inflammatory Role of Infiltrating Macrophages in Acetaminophen-Induced Liver Injury', The FASEB Journal, pp. 547–547. doi: 10.1096/fasebj.22.2_supplement.547.
28. Jaeschke, H. (2003) 'The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity', Toxicology Letters, pp. 279–288. doi: 10.1016/s0378-4274(03)00239-x.
29. Jaeschke, H. et al. (2012) 'Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity', Liver International, pp. 8–20. doi: 10.1111/j.1478-3231.2011.02501.x.
30. Jaeschke, H. (2015) 'Acetaminophen: Dose-Dependent Drug Hepatotoxicity and Acute Liver Failure in Patients', Digestive diseases , 33(4), pp. 464–471.
31. James, L. P. et al. (2007) 'Acetaminophen Protein Adducts in Children With Acute Liver Failure of Indeterminate Cause: In Reply', PEDIATRICS, pp. 419–420. doi: 10.1542/peds.2006-3449.
32. Jeevanandan, G. and Govindaraju, L. (2018) 'Clinical comparison of Kedo-S paediatric rotary files vs manual instrumentation for root canal preparation in primary molars: a double blinded randomised clinical trial', European Archives of Paediatric Dentistry, pp. 273–278. doi: 10.1007/s40368-018-0356-6.
33. J, P. C. et al. (2018) 'Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study', Clinical implant dentistry and related research, 20(4), pp. 531–534.
34. Karthiga, P., Rajeshkumar, S. and Annadurai, G. (2018) 'Mechanism of Larvicidal Activity of Antimicrobial Silver Nanoparticles Synthesized Using Garcinia mangostana Bark Extract', Journal of Cluster Science, pp. 1233–1241. doi: 10.1007/s10876-018-1441-z.
35. Khandelwal, N. et al. (2011) 'Unrecognized acetaminophen toxicity as a cause of indeterminate acute liver

failure', Hepatology, pp. 567–576. doi: 10.1002/hep.24060.
36. Lakshmi, T. et al. (2015) 'Azadirachta indica: A herbal panacea in dentistry - An update', Pharmacognosy reviews, 9(17), pp. 41–44.
37. Lancaster, E. M., Hiatt, J. R. and Zarrinpar, A. (2015) 'Acetaminophen hepatotoxicity: an updated review', Archives of Toxicology, pp. 193–199. doi: 10.1007/s00204-014-1432-2.
38. Larson, A. M. et al. (2005) 'Acetaminophen-induced acute liver failure: Results of a United States multicenter, prospective study', Hepatology, pp. 1364–1372. doi: 10.1002/hep.20948.
39. Leang, Y. et al. (2014) 'Reported ingested dose of paracetamol as a predictor of risk following paracetamol overdose', European Journal of Clinical Pharmacology, pp. 1513–1518. doi: 10.1007/s00228-014-1756-0.
40. Lee, W. M. (2004) 'Acetaminophen and the U.S. acute liver failure study group: Lowering the risks of hepatic failure', Hepatology, pp. 6–9. doi: 10.1002/hep.20293.
41. Lee, W. M. (2008) 'Acetaminophen-related acute liver failure in the United States', Hepatology Research, pp. S3–S8. doi: 10.1111/j.1872-034x.2008.00419.x.
42. Liu, Z.-X., Govindarajan, S. and Kaplowitz, N. (2004) 'Innate immune system plays a critical role in determining the progression and severity of acetaminophen hepatotoxicity', Gastroenterology, pp. 1760–1774. doi: 10.1053/j.gastro.2004.08.053.
43. Malli Sureshbabu, N. et al. (2019) 'Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases', Case reports in dentistry, 2019, p. 7046203.
44. Martin-Murphy, B. V., Holt, M. P. and Ju, C. (2010) 'The role of damage associated molecular pattern molecules in acetaminophen-induced liver injury in mice', Toxicology Letters, pp. 387–394. doi: 10.1016/j.toxlet.2009.11.016.
45. Mathew, M. G. et al. (2020) 'Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: Randomized controlled trial', Clinical oral investigations, pp. 1–6.
46. Matthews, A. (1997) 'Comparison of covalent binding of acetaminophen and the regioisomer 3′-hydroxyacetanilide to mouse liver protein', Toxicology Letters, pp. 77–82. doi: 10.1016/s0378-4274(96)03831-3.
47. McGill, M. R. et al. (2012) 'The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation', Journal of Clinical Investigation, pp. 1574–1583. doi: 10.1172/jci59755.
48. McGill, M. R., Cao, M., et al. (2014) 'Argininosuccinate synthetase as a plasma biomarker of liver injury after acetaminophen overdose in rodents and humans', Biomarkers, pp. 222–230. doi: 10.3109/1354750x.2014.897757.
49. McGill, M. R., Li, F., et al. (2014) 'Circulating acylcarnitines as biomarkers of mitochondrial dysfunction after acetaminophen overdose in mice and humans', Archives of Toxicology, pp. 391–401. doi: 10.1007/s00204-013-1118-1.
50. McGill, M. R. and Jaeschke, H. (2013) 'Metabolism and Disposition of Acetaminophen: Recent Advances in Relation to Hepatotoxicity and Diagnosis', Pharmaceutical Research, pp. 2174–2187. doi: 10.1007/s11095-013-1007-6.
51. Mehta, M. et al. (2019) 'Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases', Chemico-biological interactions, 308, pp. 206–215.
52. Menon, S. et al. (2018) 'Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism', Colloids and Surfaces B: Biointerfaces, pp. 280–292. doi: 10.1016/j.colsurfb.2018.06.006.
53. Moalem, S. (2020) The Better Half: On the Genetic Superiority of Women. Farrar, Straus and Giroux.
54. Mühl, H. and Pfeilschifter, J. (2004) 'Interleukin-18 bioactivity: a novel target for immunopharmacological anti-inflammatory intervention', European journal of pharmacology, 500(1-3), pp. 63–71.
55. Murray, K. F. et al. (2008) 'Drug-related Hepatotoxicity and Acute Liver Failure', Journal of Pediatric Gastroenterology and Nutrition, pp. 395–405. doi: 10.1097/mpg.0b013e3181709464.
56. Myers, T. G. et al. (1995) 'A Comparative Study of Mouse Liver Proteins Arylated by Reactive Metabolites of Acetaminophen and Its Nonhepatotoxic Regioisomer, 3'-Hydroxyacetanilide', Chemical Research in Toxicology, pp. 403–413. doi: 10.1021/tx00045a012.
57. Nelson, S. (1990) 'Molecular Mechanisms of the Hepatotoxicity Caused by Acetaminophen', Seminars in Liver Disease, pp. 267–278. doi: 10.1055/s-2008-1040482.
58. Panchal, V., Jeevanandan, G. and Subramanian, E. M. G. (2019) 'Comparison of post-operative pain after root canal instrumentation with hand K-files, H-files and rotary Kedo-S files in primary teeth: a randomised clinical trial', European archives of paediatric dentistry: official journal of the European Academy of Paediatric Dentistry, 20(5), pp. 467–472.
59. Pc, J., Marimuthu, T. and Devadoss, P. (2018) 'Prevalence and measurement of anterior loop of the mandibular canal using CBCT: A cross sectional study', Clinical implant dentistry and related research.

Available at: https://europepmc.org/article/med/29624863.
60. Perumalsamy, H. et al. (2018) 'In silico and in vitro analysis of coumarin derivative induced anticancer effects by undergoing intrinsic pathway mediated apoptosis in human stomach cancer', Phytomedicine: international journal of phytotherapy and phytopharmacology, 46, pp. 119–130.
61. Prabakar, J. et al. (2018) 'Comparative Evaluation of Retention, Cariostatic Effect and Discoloration of Conventional and Hydrophilic Sealants - A Single Blinded Randomized Split Mouth Clinical Trial', Contemporary clinical dentistry, 9(Suppl 2), pp. S233–S239.
62. Qiu, Y., Benet, L. Z. and Burlingame, A. L. (1998) 'Identification of the Hepatic Protein Targets of Reactive Metabolites of Acetaminophenin Vivoin Mice Using Two-dimensional Gel Electrophoresis and Mass Spectrometry', Journal of Biological Chemistry, pp. 17940–17953. doi: 10.1074/jbc.273.28.17940.
63. Rajendran, R. et al. (2019) 'Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study', Pesquisa Brasileira em Odontopediatria e Clínica Integrada, pp. 1–10. doi: 10.4034/pboci.2019.191.61.
64. Rajeshkumar, S., Kumar, S. V., et al. (2018) 'Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells', Enzyme and microbial technology, 117, pp. 91–95.
65. Rajeshkumar, S., Agarwal, H., et al. (2018) 'Brassica oleracea Mediated Synthesis of Zinc Oxide Nanoparticles and its Antibacterial Activity against Pathogenic Bacteria', Asian Journal of Chemistry, pp. 2711–2715. doi: 10.14233/ajchem.2018.21562.
66. Rajeshkumar, S. et al. (2019) 'Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through Cissus arnotiana plant extract', Journal of photochemistry and photobiology. B, Biology, 197, p. 111531.
67. Ramadurai, N. et al. (2019) 'Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial', Clinical oral investigations, 23(9), pp. 3543–3550.
68. Ramakrishnan, M., Dhanalakshmi, R. and Subramanian, E. M. G. (2019) 'Survival rate of different fixed posterior space maintainers used in Paediatric Dentistry - A systematic review', The Saudi dental journal, 31(2), pp. 165–172.
69. Ramesh, A. et al. (2018) 'Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study', Journal of periodontology, 89(10), pp. 1241–1248.
70. R, H. et al. (2020) 'CYP2 C9 polymorphism among patients with oral squamous cell carcinoma and its role in altering the metabolism of benzo[a]pyrene', Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, pp. 306–312. doi: 10.1016/j.oooo.2020.06.021.
71. Ring-Larsen, H. and Palazzo, U. (1981) 'Renal failure in fulminant hepatic failure and terminal cirrhosis: a comparison between incidence, types, and prognosis', Gut, pp. 585–591. doi: 10.1136/gut.22.7.585.
72. Samuel, S. R. (2021) 'Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life?', International journal of paediatric dentistry / the British Paedodontic Society [and] the International Association of Dentistry for Children, 31(2), pp. 285–286.
73. Samuel, S. R., Acharya, S. and Rao, J. C. (2020) 'School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial', Journal of public health dentistry, 80(1), pp. 51–60.
74. Sandhu, N. and Navarro, V. (2020) 'Drug-Induced Liver Injury in GI Practice', Hepatology communications, 4(5), pp. 631–645.
75. Sharma, P. et al. (2019) 'Emerging trends in the novel drug delivery approaches for the treatment of lung cancer', Chemico-biological interactions, 309, p. 108720.
76. Sridharan, G. et al. (2019) 'Evaluation of salivary metabolomics in oral leukoplakia and oral squamous cell carcinoma', Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology, 48(4), pp. 299–306.
77. Thulin, P. et al. (2014) 'Keratin-18 and microRNA-122 complement alanine aminotransferase as novel safety biomarkers for drug-induced liver injury in two human cohorts', Liver International, pp. 367–378. doi: 10.1111/liv.12322.
78. Varghese, S. S., Ramesh, A. and Veeraiyan, D. N. (2019) 'Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students', Journal of dental education, 83(4), pp. 445–450.
79. Vijayashree Priyadharsini, J. (2019) 'In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens', Journal of periodontology, 90(12), pp. 1441–1448.
80. Vijayashree Priyadharsini, J., Smiline Girija, A. S. and Paramasivam, A. (2018) 'In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species', Archives of oral biology, 94, pp. 93–98.

81. Vishnu Prasad, S. et al. (2018) 'Report on oral health status and treatment needs of 5-15 years old children with sensory deficits in Chennai, India', Special care in dentistry: official publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry, 38(1), pp. 58–59.
82. Wahab, P. U. A. et al. (2018) 'Scalpel Versus Diathermy in Wound Healing After Mucosal Incisions: A Split-Mouth Study', Journal of oral and maxillofacial surgery: official journal of the American Association of Oral and Maxillofacial Surgeons, 76(6), pp. 1160–1164.
83. Wong, A. et al. (2020) 'Efficacy of a two bag acetylcysteine regimen to treat paracetamol overdose (2NAC study)', EClinicalMedicine, 20, p. 100288.
84. Xie, Y. et al. (2014) 'Mechanisms of acetaminophen-induced cell death in primary human hepatocytes', Toxicology and Applied Pharmacology, pp. 266–274. doi: 10.1016/j.taap.2014.05.010.
85. Xie, Y. et al. (2015) 'Time course of acetaminophen-protein adducts and acetaminophen metabolites in circulation of overdose patients and in HepaRG cells', Xenobiotica, pp. 921–929. doi: 10.3109/00498254.2015.1026426.
86. Yang, X. et al. (2015) 'Potential of extracellular microRNAs as biomarkers of acetaminophen toxicity in children', Toxicology and Applied Pharmacology, pp. 180–187. doi: 10.1016/j.taap.2015.02.013.
87. Yoon, E. et al. (2016) 'Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update', Journal of clinical and translational hepatology, 4(2), pp. 131–142.