

# Analysis of Biological Activity Like Antioxidant, Antimicrobial, and DNA Damage of Paracetamol

Kalpana Barasker, Neelu Jain\*, Parul Jain, K. Gour

Received: 19 October 2023 / Received in revised form: 16 February 2024, Accepted: 22 February 2024, Published online: 15 March 2024

## Abstract

Paracetamol has been employed over an extensive duration as an analgesic and antipyretic agent. Subsequently, there has been a notable escalation in the incidence of poisoning with paracetamol emerging as the predominant substance in cases of self-poisoning, associated with elevated fatality and morbidity rates. While the utilization, misuse, and strategies for mitigating paracetamol toxicity are under examination, serious consideration should be given to altering the drug's legal classification-potentially transitioning it to a prescription-only status, given the inherent risks. The most effective approach to quantifying the uneven distribution of resultant liver injury involves correlating plasma enzyme activity in the affected liver regions. Although liver glutathione levels decrease similarly with 3% casein + phenobarbitone and yeast diets, animals fed yeast exhibit heightened sensitivity to paracetamol despite lower cytochrome P-450 levels. The addition of methionine to the oral dose of paracetamol proves preventive against fatalities and liver damage, suggesting a potentially valuable strategy for enhancing the safety of paracetamol administration, particularly in cases of potential overdose.

**Keywords:** Glutathione, Toxicity, Potential, Analgesic, Antipyretic, Methionine

## Introduction

Paracetamol reduces body temperature in several ways. With a strong safety record, paracetamol is a 4-hydroxy acetanilide in terms of chemistry. Many attempts were undertaken to modify the composition of this medicine like hydroxyl and acetamide group after it showed promise as effects of analgesic and antipyretic

activities. The goals were to improve the drug's efficacy, cover up its bitter taste, and lessen its toxicity. One prodrug that is readily available and effective when administered parenterally is propacetamol, which is produced from paracetamol. The hepatotoxicity of paracetamol ester prodrugs including sulfur-containing amino acids, such as methionine, N-acetyl cysteine, and cysteine, was negligible in comparison to the parent drug (Stella, 2020). Antibacterial, antioxidant, and cancer-preventive qualities were also demonstrated by a wide range of compounds with diverse characteristics, including thymol, triazole ring, chalcones, nucleoside analogs, metal complexes, and hybrids with the aryl-imidazolidinyl ring. Nowadays, paracetamol is used in a variety of ways for analgesia, either by itself or in conjunction with other medications (typically opiates) or in various combinations for its analgesic and antipyretic qualities, such as cold "cures." A medication ought to be both effective and bearable, meaning it shouldn't have any negative or overbearing side effects. The tolerance of aspirin, ibuprofen, and paracetamol is compared in just one randomized controlled study. Six According to this study, paracetamol did not tolerate as well as ibuprofen, but it did endure it better than aspirin. Numerous studies conducted since 1990 have demonstrated that paracetamol is a useful antipyretic; yet, its analgesic efficacy in comparison to other medications, such as ibuprofen (another commonly used and easily accessible analgesic), is not consistently high Józwiak-Bebenista and Nowak (2014). Overdosing on paracetamol is a major reason for hospital admissions, however, the prognosis is usually favorable, and severe liver damage is rare. A prothrombin time >18 s, an alanine transaminase >45 IU/l, and encephalopathy were considered severe liver injury. Even though 6.9% of patients had these symptoms, all of them recovered with supportive treatment, and none of them required liver transplantation Graham and Scott (2005).

The development, Biosynthesis, and evaluation of NSAID medicines with improved efficacy and fewer adverse side effects is one of the primary challenges of the contemporary period Cleydson B R Santos *et al.* (2023). Using <sup>1</sup>H and <sup>13</sup>C NMR, the structures of two paracetamol were clarified. By adding more methyl to an acetamide molecule's alkyl position, several derivatives were created. The goal of the methylation changes at the phenyl ring and in large numbers are identified, for example—is to enhance the resistance in cyclooxygenase activity of Acetaminophen and its derivatives were created using acyl chloride or anhydride in conventional acylation procedures Dittert *et al.* (1968). Predicting pharmacokinetics and toxicological

**Kalpana Barasker, Neelu Jain\***

Department of Chemistry, Sri Satya Sai University of Technology and Medical Sciences, Sehore, M.P, India.

**Parul Jain**

Department of Chemistry, S.V Polytechnic College, Bhopal, India.

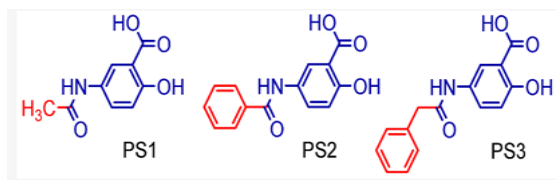
**K. Gour**

Department of Chemistry, Priyadarshini J.L College of Engineering, Nagpur, Maharashtra, India.

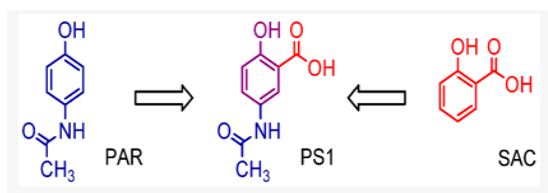
\*E-mail: Jneelu586@gmail.com



features was done by computational methods. When it comes to its anti-nociceptive action, benzoic acid has less painful activity than acetaminophen and Acetaminophen. Nonetheless, the recommended substances aid in the creation of fresh, safer derivative possibilities and could even be more potent than 5-acetamido-2-hydroxy benzoic acid. Therefore, more research is required to assess the various pharmacological activities, potential toxicity of any putative metabolites, and prospective applications in the treatment of pain and inflammation.



**Figure 1.** Benzoic acid (5-acetamido-2-hydroxy) and its derivatives (PS1, PS2, PS3)



**Figure 2.** 5-acetamido-2-hydroxy benzoic acid's (PS1) chemical structure

In the formalin test, Acetaminophen demonstrated peripheral anti-nociceptive activity as demonstrated by a decrease in acetic acid-induced abdominal writhing behavior and an anti-inflammatory impact during the inflammatory phase but not the neurogenic phase. This analgesic effect exhibits a superior activity 10 to 25 times more effective than its antecedents when compared to its cousins Chan and Graham (2004). Furthermore, in the hot-plate test, 5-acetamido-2-hydroxy benzoic acid elicited central anti-nociceptive activity throughout both the neurogenic and inflammatory phases of the formalin test being successful in lessening the production of edema brought on by croton oil or carrageenan. This is typically their first attempt at synthesizing a pharmaceutically active ingredient, which always inspires high hopes and determination. Furthermore, although paracetamol (acetaminophen) is an analgesic that is commonly used to treat less to more severe pain, moreover, it is frequently accessible in family pharmacies, it can also be a suitable topic to discuss regarding paracetamol (acetaminophen) toxicity because acute overdoses are very common and can result in serious liver damage.

## Materials and Methods

### *Analysis of 5-Acetamido-2-Hydroxy Benzoic Acid synthesis and Derivatives*

Acylation reaction was done with the help of amine and acyl chloride through a nucleophilic addition reaction. All derivatives were identified with the help of the NMR technique.

### *Analysis of Toxicity Risk Assessment*

Scientists make decisions about the likelihood that human toxicity may manifest itself through the process of risk assessment. Risk assessment is stated as involving any or all of the following parts by the National Research Council (1983). These authorities are responsible for the management of dose-response assessment, characterization of risk, and drug exposure assessment (Liu *et al.*, 2022). All things considered; this format could be modified for the process of determining the risk of developmental toxicity to humans. The identification of hazards for developmental toxicity and other noncancer health effects, however, is typically carried out in tandem with a review of dose-response relationships in practice, as frequently influences the hazard assessment (Kimmel, 1996).

### *Analysis of Antipyretic Activity*

Elahe Mirrasekhian *et al.* (2018) found that the activation of TRPA1 by paracetamol's electrophilic metabolites is most likely what causes paracetamol-induced hypothermia. Paracetamol's antipyretic action in mice is reliant on cyclooxygenase activity suppression, which includes the production of pyrogenic prostaglandin E2.

### *Analysis of Paracetamol –Mechanisms of Action*

- COX-dependent central mechanism
- CYCLO OXYGENASES mechanism

### *Analgesic Activity*

Adults who have acute pain of any kind, such as body aches, headache and muscle pain, menstrual pain, bone pain, and surgery-related pain, can use paracetamol to get relief. Adults can take 500 mg tablets orally every 6 hours (Ayoub, 2021).

### *Antimicrobial Activity*

It is the time to inhibit the growth of bacteria and fungus. It is the interaction of microbes and antimicrobial medicines that can be comprehended through the application of this technology. One can observe a concentration- or time-dependent antibacterial action using the time-kill test. A complete description and standardization of this microorganism test are included in the CLSI M26-A publication.

### *Antioxidant Activity*

We measured total antioxidant capacity using assay kits (lab science, TX, USA). The OD value of 520 nm was measured using a Biotek Synergy multimode microplate reader (Hassan *et al.*, 2020).

## Results and Discussion

### *Acetamido-2-Hydroxy Benzoic Acid Synthesis and Derivatives*

During the synthesis outlined, 5-amino-2-hydroxy benzoic acid (A) is converted into 5-acetamido-2-hydroxy benzoic acid and its derivatives by N-acylation reactions with either benzoyl chloride

(PS2) or phenylacetyl chloride (PS3), or by acetylation (PS1). As a catalyst, potassium carbonate is used in conjunction with either water or ethyl acetate as the solvent in this procedure (Pham-Huy *et al.*, 2008). It is explained below in detail.

#### Acetylation (PS1)

Reagent: 5-amino-2-hydroxy benzoic acid (A)  
 Reaction: Acetylation involves the introduction of an acetyl group (-COCH<sub>3</sub>) to the amino group of the starting material.  
 Solvent: Water or ethyl acetate  
 Catalyst: Potassium carbonate  
 Resulting Product: 5-acetamido-2-hydroxy benzoic acid

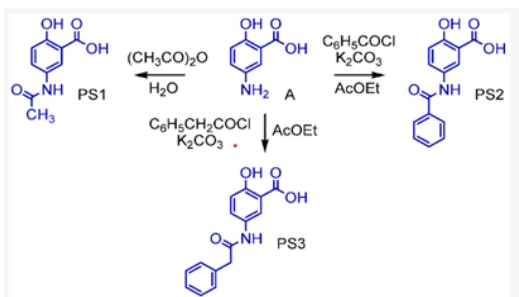
#### N-Acylation with Benzoyl Chloride (PS2)

Reagent: 5-acetamido-2-hydroxy benzoic acid (product from PS1)  
 Reaction: N-acylation refers to the attachment of an acyl group to the nitrogen atom of the amino group in the starting material.  
 Solvent: Water or ethyl acetate  
 Catalyst: Potassium carbonate  
 Resulting Product: Derivative of 5-acetamido-2-hydroxy benzoic acid with a benzoyl group attached to the amino nitrogen.

#### N-Acylation with Phenylacetyl Chloride (PS3)

Reagent: 5-acetamido-2-hydroxy benzoic acid (product from PS1)  
 Reaction: Similar to PS2 but utilizing phenylacetyl chloride as the acylating agent.  
 Solvent: Water or ethyl acetate  
 Catalyst: Potassium carbonate  
 Resulting Product: Derivative of 5-acetamido-2-hydroxy benzoic acid with a phenylacetyl group attached to the amino nitrogen.

In summary, the process involves sequentially acetylating the amino group and then N-acylating the resulting product using either benzoyl chloride or phenylacetyl chloride. The reactions are conducted in the presence of potassium carbonate as a catalyst, and the choice of solvents includes water or ethyl acetate. The specific details of the reaction conditions, such as temperature and reaction times, may need to be optimized in the laboratory for the best yield and purity of the desired products. **Figure 3** Illustration provide a comprehensive overview of the methods employed in the synthesis of 5-acetamido-2-hydroxy benzoic acid and its derivatives, highlighting the crucial steps involving acetylation and N-acylation reactions (PS1, PS2, PS3).



**Figure 3.** Methods for synthesizing 5-acetamido-2-hydroxy benzoic acid and its derivatives

#### Toxicity Risk Assessment

An essential component of therapeutic medication action is molecular weight; when it rises above a threshold, the compounds become bulkier, which impacts the therapeutic effect of the medicine. Certain pharmacological compounds defy this requirement; in fact, some of them even show favorable liposolubility features while having a molecular weight larger than the threshold value (Turones *et al.*, 2023).

Urine contains the conjugate of glucuronide and sulfate, which is the primary byproduct of paracetamol metabolism. The conversion of N-acetyl-p-amine-benzoquinone in small quantities is linked to hepatotoxicity (Ahmed *et al.*, 2023). By conjugating, N-acetyl-p-benzoquinone imine with N-Glutathione is detoxified in therapeutic proportions; however, damage to the liver and kidneys may occur if the intracellular glutathione stores that provide protection are exhausted. Despite using a different method than glutathione repletion, N-acetylcysteine appears to improve mortality in liver failure caused by paracetamol. Methionine and N-acetylcysteine both restore glutathione reserves in the kidney and liver. While N-acetylcysteine is accessible orally and intravenously, methionine is administered orally Chandan *et al.* (2021).

There is virtually little information on cardiotoxicity after paracetamol intoxication, despite the well-studied hepatotoxicity after the same. It's possible that paracetamol-induced cardiotoxicity was disregarded (Ralapanawa *et al.*, 2016), despite the fact that clinically significant cases have rarely been reported. A few papers have discussed the potential for a paracetamol overdose to be immediately cardiotoxic. Since typical clinical management does not include serial electrocardiograph (ECG) monitoring, the true incidence remains unknown (Bessede *et al.*, 2022). Concerns have also been raised about the inexplicable deaths linked to paracetamol poisoning, some of which happen within 24 hours of use. The possibility that some of these have cardiac origins cannot be ruled out. Dysrhythmias and other ECG abnormalities, notably of the ST segment or T wave, are commonly detected in comatose encephalopathic individuals, even though ST/T wave abnormalities have been recorded in non-encephalopathic people. Before acute hepatic failure is verified, abrupt, unexplained death from paracetamol overdose can occur even in the total lack of any histopathological indication of hepatic necrosis.

Based on postmortem findings of cardiac necrosis, especially in the sub-endocardium, direct toxic myocarditis has been hypothesized. There have been documented cases of toxic myocarditis and abrupt cardiac necrosis brought on by paracetamol overdose. In encephalopathic individuals, the origin of the ECG abnormalities is most likely complex and at least partially associated with significant metabolic abnormalities. These, however, cannot be connected to individuals who have less liver damage or who pass away suddenly from a paracetamol overdose at an early stage. Armour *et al.* (2016) advised obtaining an ECG at the time of admission and monitoring it daily to determine if a significant overdose had occurred as a result. Moreover, they said that regardless of the paracetamol plasma content or the amount of time

that has passed after ingestion, therapy with a conventional acetylcysteine infusion should be investigated if ST/T wave abnormalities or dysrhythmia are evident. A review of the literature revealed that very little research had been done to determine the cardiotoxicity of paracetamol, an issue that appears to have been overlooked (Abedi *et al.*, 2020).

#### Antipyretic Activity

Paracetamol is widely used to relieve pain and fever globally, it has garnered prolonged attention for its safety and mechanism of action. Despite its extensive usage, uncertainties persist regarding its application and pathways influencing its analgesic and antipyretic effects. Current understanding posits paracetamol as a multifaceted medication, with its actions attributed to various metabolic pathways. Implicated in its mechanism are the endocannabinoid system, Cyclooxygenase, and serotonergic pathways. Additionally, paracetamol impacts the activity of TRP channels or T-type calcium channels. It also modulates L-arginine, influencing the biosynthesis of nitrous oxide (NO). However, certain effects lack conclusive evidence. This study aims at the current understanding of paracetamol's mechanism of action, emphasizing safety concerns.

#### Paracetamol –Mechanisms of Action

##### COX-Dependent Central Mechanism

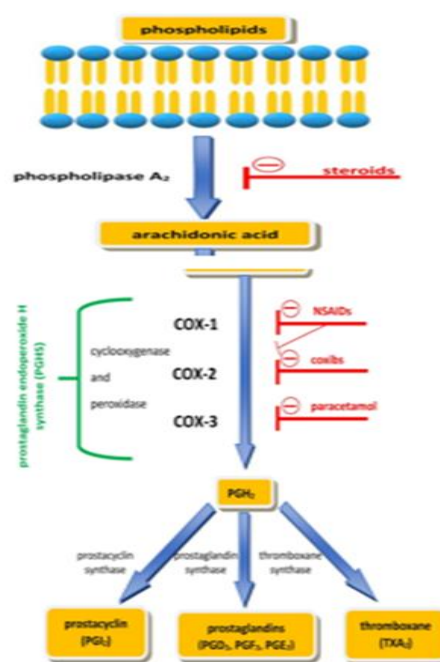
Speculations about the last identified COX-1 AND COX-2 isoenzyme's impacts on the central nervous system persisted even after the idea of the paracetamol action related to COX-3 was demonstrated to be unfounded. To begin with, when compared to traditional COX-1 inhibitors such as aspirin, paracetamol has a lower rate of side effects and less anti-inflammatory efficacy. Consequently, a particular prostaglandin inhibitor in the central nervous system may be paracetamol Ayoub (2021). Research indicated that paracetamol might be used as an analgesic without blocking the generation of prostaglandins, or that it could lower biosynthesis of prostaglandin in the brain than spleen which is ten times lesser. Second, research by Graham and Scott demonstrated the paracetamol, later studies by Hinz and Brune (2012) as well as other studies indicate that paracetamol is a preferred constraint of isoenzyme COX-2 in conditions where the surrounding medium's peroxide content was low (De Coster *et al.*, 2020). The isoenzyme COX activity which is peroxide dependent explains the paracetamol-induced suppression of inflammation and lack of platelet activity. Furthermore, Kalgutkar *et al.* demonstrated that in LPS-stimulated macrophages, N-arachidonoylphenolamine (AM404), a metabolite of paracetamol, inhibited COX-1 and COX-2. Saliba *et al.* (2017) presented novel and important insights into how AM404 inhibited COX-1 and COX-2 activity in activated microglia, hence reducing prostaglandin (PGE<sub>2</sub>, PGD<sub>2</sub>) production. This idea relates to AM404's anti-inflammatory properties.

The primary mediators of inflammatory pain are prostaglandins. The term "cyclooxygenases" refers to the enzymes that produce these mediators. Cyclooxygenase (COX-1) was first discovered by John Vane in 1971. This finding improved our understanding of aspirin's mode of action. Aspirin is also used like paracetamol for

the removal of pain and fever since 1899. The second cyclooxygenase (COX-2) was later identified by Xie *et al.* at Brigham Young University in Daniel Simmons's lab in 1991. Notably, the structures of the previously reported COX-1 and COX-2 enzymes remained mostly unchanged. However, their clinical importance varies.

#### Cyclooxygenases Mechanism

The PGHS is a bifunctional enzyme of prostaglandin endoperoxide synthase also comprises cyclooxygenases and transforms arachidonic acid (AA) into prostaglandin H<sub>2</sub> (PGH<sub>2</sub>). Arachidonic acid is extremely specifically oxygenated in the 11R configuration to start the prostaglandin (PG) COX production process. This is followed by a 15S oxygenation to generate PGG<sub>2</sub>. After that, PGG<sub>2</sub> is decreased to PGH<sub>2</sub> by POX action. Prostacyclin (PGI<sub>2</sub>), thromboxane, and prostaglandins families are further produced from prostaglandin H by a variety of tissue enzymes Hinz and Brune (2012).



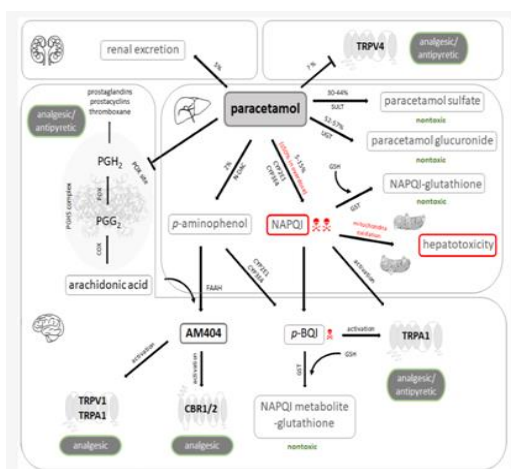
**Figure 4.** Cyclooxygenase mechanism

Treatment with nonsteroid anti-inflammatory drugs (NSAIDs) mostly involves blocking COX-1 and COX-2 in different ratios. These isoenzymes are expressed in many body organs like the kidney and mucosal layer of the stomach. **Figure 4** explain the cyclooxygenase mechanism, depicting the conversion of arachidonic acid to prostaglandin H<sub>2</sub>, a pivotal step in eicosanoid synthesis. Consequently, the generation of protective prostaglandins E<sub>2</sub> (PGE<sub>2</sub>) and I<sub>2</sub> (PGI<sub>2</sub>) in the stomach mucosa is decreased by repeated high doses of NSAIDs, which may result in gastrointestinal adverse effects such as gastric ulcers (Begum *et al.*, 2023). Conversely, COX-2 only becomes active while inflammation is still present. As a result, selective COX-2 medications (coxibs) lessen the adverse effects of treating patients with COX-1-dependent medications.

### Analgesic Activity

Acetaminophen, sometimes known as paracetamol is used frequently and in wide-area medication globally. Contempt, its widespread use, and its lengthy history of use, questions persist about the safe use of this medication and find out. It is currently believed that it is a multiuse drug whose analgesic and antipyretic actions are caused by at least a few metabolic pathways (Srivastava *et al.*, 2020). It is considered that paracetamol impacts the potassium channel, and transient receptors, and also influences the pathway of L-Arginine biosynthesis that results in the production of nitric oxide (NO). Not all of these impacts, nevertheless, have received solid confirmation. Therefore, the goal of our study was to present a summary of the state of knowledge on the metabolic mechanism of Acetaminophen, with an emphasis on safety concerns. The activation of descending serotonergic pathways contributes to the primary analgesic effect of Acetaminophen. Its primary method of action is unclear; it may work by blocking the manufacture of prostaglandins (PGs) or by influencing cannabinoid receptors through an active metabolite.

Although pregnant women frequently take paracetamol to manage pain and fever, there is growing worry that this medication may result in ADHD and Autism related genetic disorders in the unborn child. An increasing body of epidemiological research indicates that taking paracetamol while pregnant increases relative risks for these diseases by about 25% on average. Dise-related effects are not adequate to completely consider the confounders that cannot be measured like genetic factors. Only a portion of this issue has been studied experimentally (Dinesh, 2020). When paracetamol was administered to adult animals, the analgesic effect was lessened, and when the medication was given to newborn mice at or before day 10, the mice's adult locomotor activity changed in reaction to their new surroundings. The offspring of pregnant rats who were gavaged with paracetamol also displayed altered behavior. What chemical mechanisms might be causing these effects is unknown.



**Figure 5.** Mechanism of Action and Different Activities of Paracetamol

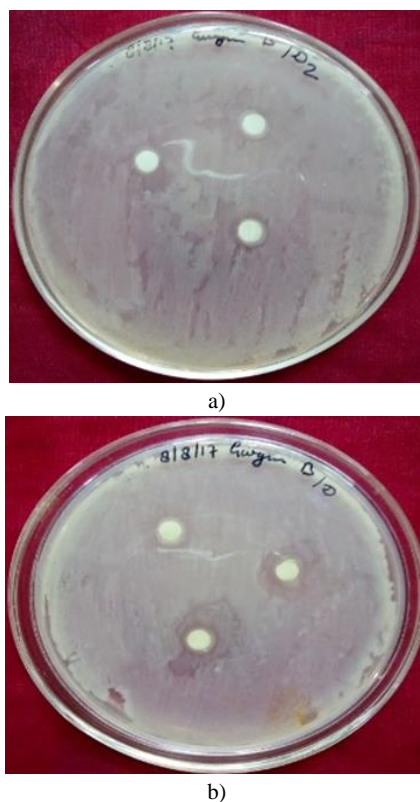
**Figure 5** shows of overview of Paracetamol's Mechanism of Action and Diverse Activities, encompassing analgesic and

antipyretic effects, potential impacts on the endocannabinoid system, serotonergic pathways, and modulation of cyclooxygenases (COX-1, COX-2, and COX-3). There are several different pharmacologic actions of paracetamol. It reduces prostaglandin production by competitively blocking the pathway of prostaglandin H<sub>2</sub> synthase. On the other hand, N-arachidonoyl phenolamine blocks cannabinoid receptor signaling and temporarily activates vanilloid-subtype 1 receptors. The compound N-acetyl-p-benzo quinone imine is responsible for peroxide stress and reduction of glutathione in the brain which is important for liver damage following overdose which causes liver damage at the threshold dose. Taking into account the prevalence of Acetaminophen usage among pregnant women and the scarcity of safe substitutes (Saliba *et al.*, 2017).

Acetaminophen is also known as paracetamol which is mostly and often used medications for pain relief by small children and pregnant women worldwide. Paracetamol was thought to be safe to take during pregnancy until recently. Nonetheless, a growing body of evidence—albeit contentious—indicates that pregnant women who take it may cause their children to develop genetic abnormalities like ADHD and Autism.

### Antimicrobial Activity

The antimicrobial test reveals an antibacterial effect that is either concentration- or time-dependent. The CLSI M26-A publication contains comprehensive standardization and documentation of this test for microorganisms. Four test tubes are used which consist of microbial suspension of 1, 2, and 3 CFU/mL. The third tube is assumed to represent the growth control, whereas the chemical or extract under study is normally present in the first and second tubes at 0.25, 0.50, and 0.75 MIC. The percentage of dead cells is then determined concerning the cleared zone obtained or the growth of microbes controlled by the antimicrobial agents. Usually, a 90% lethality percentage for six hours or a 99.9% lethality percentage for twenty-four hours is obtained for the bactericidal action (Frei *et al.*, 2020). This approach may also be used to determine whether drug combinations are more antagonistic or synergistic. The response (MIC) is quantified in terms of inhibitory concentration and zone of inhibition. In benzoic acid, the lowest zone of inhibition for *Bacillus subtilis* at antimicrobial dilution concentrations is around 23 mm and 19 mm, respectively; in ethanol extract, the values are 10 mm and 7 mm, respectively. **Figure 6** Demonstrate Paracetamol's Antimicrobial Activity Against *Bacillus subtilis*, illustrating its inhibitory effects on bacterial growth. **Table-1** depict the Inhibition Zones (mm) Against Pathogenic Microbes, illustrating the measured diameters of clear zones around antimicrobial agents, indicating their effectiveness in inhibiting microbial growth.



**Figure 6.** Antimicrobial activity of paracetamol in *Bacillus subtilis*

**Table 1.** Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubation

S.No.	Solvent	Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubation ( <i>Bacillus subtilis</i> )		
		0.75mg	0.5mg	0.25mg
1	Benzoic acid	19 ± 0.6	17 ± 0.6	14 ± 0.6
2	Ethanol	7 ± 0.2	8 ± 0.4	5 ± 0.2

#### Antioxidant Activity

Paracetamol, a widely used analgesic and antipyretic drug, exhibits nephrotoxicity at elevated doses. At therapeutic levels, approximately 85% of paracetamol undergoes phase II conjugation in the liver, producing sulfate and glucuronide metabolites eliminated by the kidneys. About 10% undergoes phase I oxidation, primarily mediated by cytochrome 2E1, forming NAPQI. Under normal conditions, NAPQI conjugates with glutathione to form non-toxic metabolites. However, at high doses, paracetamol metabolism triggers potential toxicity mechanisms (Chen *et al.*, 2019). Excessive metabolite production depletes glutathione, leading to the accumulation of reactive species. Electrophilic intermediates form adducts with cellular proteins, activating caspases and lysosomal enzymes, causing liver cell necrosis, hepatocellular content leakage, and liver failure. NAPQI-induced free radicals play a pivotal role in paracetamol toxicity, promoting lipid peroxidation and releasing products such as MDA,

reflecting tissue damage. Additionally, NAPQI-induced oxidative stress can reduce the activity of antioxidant enzymes like SOD and catalase.

Paracetamol, commonly used for its analgesic properties, poses a significant risk of toxicity, with the liver being the primary organ affected in overdose cases. However, recent studies have suggested potential cardiotoxic effects following paracetamol overdose, expanding our understanding beyond hepatic implications. One proposed mechanism involves the transformation of paracetamol into N-acetyl-p-benzoquinonimine, a toxic metabolite (Akay and Tezel, 2020). This metabolite, when not adequately neutralized by glutathione, acts as a direct toxin on the myocardium. The resulting damage may contribute to both hepatic injury and myocardial damage. Covalent binding of paracetamol to proteins in the heart and liver has been observed in studies, altering their structure and function. This modification could potentially lead to tissue damage and trigger the production of cytokines, exacerbating the impact on these organs. Additionally, a theory suggests that paracetamol-induced ischemia may be linked to the depletion of sulfhydryl groups. Acetaminophen can deplete these groups, disrupting the synthesis of nitric oxide and potentially leading to coronary ischemia. The functional coronary ischemia arises from the inhibition of endothelium-derived vascular relaxing factor, further compounding the potential impact of sulfhydryl deficiency.

In summary, the cardiotoxicity associated with paracetamol overdose is multifaceted. The direct toxic action of the metabolite N-acetyl-p-benzoquinonimine, covalent binding to proteins, and disruption of sulfhydryl groups all contribute to the complex interplay of mechanisms leading to myocardial injury. Understanding these processes is crucial for developing targeted interventions and improving outcomes in cases of paracetamol toxicity (Shertzer *et al.*, 2008). The genesis of metabolic disruptions, including hyperkalemia, metabolic acidosis, and elevated blood fatty acid levels resulting from hepatic failure, is a commonly recognized explanation for cardiotoxicity in paracetamol overdose. Previous reports consistently link hepatotoxicity to instances of paracetamol poisoning that have caused harm to heart tissue. Lesna *et al.* (2015) noted that cardiac arrest cases were always associated with hepatotoxicity, suggesting that metabolic disruptions induced by hepatic failure contribute to cardiac tissue damage in paracetamol overdose. Ohtani *et al.* (2020) reported a case where hepatic function tests normalized while heart failure occurred two weeks after hepatic injury, and serum paracetamol levels were not elevated. This observation implies that cardiac toxicity may develop later, even in cases without initial symptoms of dysfunction or damage to heart tissue due to hepatic disease. Mi Jin and colleagues investigated paracetamol-induced cytotoxicity and gene expression alterations in H<sub>9</sub>C<sub>2</sub> cell cardiomyocyte cultures. Paracetamol administration to cardiomyocytes may reveal upregulation and DNA damage manifestation in several genes associated with oxidative stress, DNA damage, and apoptosis. The investigation further demonstrated that paracetamol decreased the vitality of H<sub>9</sub>C<sub>2</sub> cells in a dose- and time-dependent manner. In a separate study, explored the cardiotoxicity and hepatotoxicity (Shertzer *et al.*, 2008) of various drugs using newborn rat heart cells and rat hepatocytes. The results showed that significant doses

of paracetamol exhibited cardiotoxic effects (Day, 2021). These comprehensive studies shed light on the intricate relationship between paracetamol overdose, hepatic failure, and subsequent cardiotoxicity, providing insights into the underlying mechanisms and potential pathways leading to heart tissue damage.

## Conclusion

Due to its extensive use as an over-the-counter pain reliever and antipyretic, paracetamol has become a regular component of sewage water, contributing to its presence in human urine samples in affluent nations. This widespread occurrence in aquatic habitats has prompted research into the effects of even modest doses of paracetamol on distantly evolved marine organisms. Investigations reveal documented alterations in DNA methylation patterns, behavior, development, and enzyme activity in these organisms.

Paracetamol, a widely utilized analgesic for both acute and chronic pain, presents a challenge in understanding the precise pathways responsible for its analgesic effects due to its intricate metabolism. Initially believed to provide analgesia by inhibiting the activity of cyclooxygenase enzymes, recent research sheds light on the multifaceted impact of paracetamol on marine ecosystems and raises concerns about its potential consequences on the health and behavior of marine organisms.

**Acknowledgments:** This work is done at Sri Satya Sai University of Technology and Medical Sciences, Sehore (MP).

**Conflict of interest:** None

**Financial support:** None

**Ethics statement:** I take the responsibility for my actions, which I intend to keep. This research conducted in accordance with UGC guidelines and any person or animals are not harmed intentionally.

## References

- Abedi, F., Razavi, B. M., & Hosseinzadeh, H. (2020). A review on gentisic acid as a plant derived phenolic acid and metabolite of aspirin: Comprehensive pharmacology, toxicology, and some pharmaceutical aspects. *Phytotherapy Research*, 34(4), 729-741. doi:10.1002/ptr.6573
- Ahmed, H. M., Shehata, H. H., Mohamed, G. S., Abo-Gabal, H. H., & El-Daly, S. M. (2023). Paracetamol overdose induces acute liver injury accompanied by oxidative stress and inflammation. *Egyptian Journal of Chemistry*, 66(3), 399-408.
- Akay, C., & Tezel, U. (2020). Biotransformation of Acetaminophen by intact cells and crude enzymes of bacteria: A comparative study and modelling. *Science of the Total Environment*, 703, 134990. doi:10.1016/j.scitotenv.2019.134990
- Ayoub, S. S. (2021). Paracetamol (acetaminophen): A familiar drug with an unexplained mechanism of action. *Temperature*, 8(4), 351-371.
- Begum, S., Harisree, G. P., & Anjum, M. R. (2023). A short review on biological activities of paracetamol derivatives. *International Journal of Pharmaceutical Sciences and Nanotechnology*, 16(1), 6309-6324. Available from: <https://www.ijpsnonline.com/index.php/ijpsn/article/view/2507>
- Bessede, A., Marabelle, A., Guégan, J. P., Danlos, F. X., Cousin, S., Peyraud, F., Chaput, N., Spalato, M., Roubaud, G., Cabart, M., et al. (2022). Impact of acetaminophen on the efficacy of immunotherapy in cancer patients. *Annals of Oncology*, 33(9), 909-915. doi:10.1016/j.annonc.2022.05.010
- Chan, F. K. L., & Graham, D. Y. (2004). Prevention of non-steroidal anti-inflammatory drug gastrointestinal complications—review and recommendations based on risk assessment. *Alimentary Pharmacology & Therapeutics*, 19(10), 1051-1061.
- Chandan, J. S., Zemedikun, D. T., Thayakaran, R., Byne, N., Dhalla, S., Acosta-Mena, D., Gokhale, K. M., Thomas, T., Sainsbury, C., Subramanian, A., et al. (2021). Nonsteroidal antiinflammatory drugs and susceptibility to COVID-19. *Arthritis & Rheumatology*, 73(5), 731-739.
- Chen, J., Lu, W., Chen, H., Bian, X., & Yang, G. (2019). A new series of salicylic acid derivatives as non-saccharide  $\alpha$ -glucosidase inhibitors and antioxidants. *Biological and Pharmaceutical Bulletin*, 42(2), 231-246.
- Day, M. (2020). Covid-19: Ibuprofen should not be used for managing symptoms, say doctors and scientists. *British Medical Journal*, 368, m1086.
- De Coster, O., Forget, P., De Mey, J., Van Schuerbeek, P., & Poelaert, J. (2020). Identification of the cerebral effects of paracetamol in healthy subjects: An fMRI study. *British Journal of Pain*, 14(1), 23-30. doi:10.1177/2049463719854483
- Dinesh, V. B. (2020). Synthesis, characterization and biological activity studies of mixed ligand complexes of copper with paracetamol and amino acids. *International Journal of Grid and Distributed Computing*, 13(2), 154-161.
- Dittert, L. W., Caldwell, H. C., Adams, H. J., Irwin, G. M., & Swintosky, J. V. (1968). Acetaminophen prodrugs I. Synthesis, physicochemical properties, and analgesic activity. *Journal of Pharmaceutical Sciences*, 57(5), 774-780.
- Frei, A. (2020). Metal complexes, an untapped source of antibiotic potential? *Antibiotics*, 9(2), 90.
- Graham, G. G., & Scott, K. F. (2005). Mechanism of action of paracetamol. *American Journal of Therapeutics*, 12(1), 46-55.
- Hassan, F., Al-Yasi, H., Ali, E., Alamer, K., Hessini, K., Attia, H., & El-Shazly, S. (2020). Mitigation of salt-stress effects by moringa leaf extract or salicylic acid through motivating antioxidant machinery in damask rose. *Canadian Journal of Plant Science*, 101(2), 157-165. doi:10.1139/cjps-2020-0127
- Hinz, B., & Brune, K. (2012). Paracetamol and cyclooxygenase inhibition: is there a cause for concern? *Annals of the Rheumatic Diseases*, 71(1), 20-25.

- Jóźwiak-Bebenista, M., & Nowak, J. Z. (2014). Paracetamol: Mechanism of action, applications and safety concern. *Acta Poloniae Pharmaceutica*, 71(1), 11-23.
- Kimmel, C. A. (1996). Developmental toxicity risk assessment: consensus building, hypothesis formulation, and focused research. *Drug Metabolism Reviews*, 28(1-2), 85-103. doi:10.3109/03602539608993993
- Kralova Lesna, I., Petras, M., Cejkova, S., Kralova, A., Fronck, J., Janousek, L., Thieme, F., Tyll, T., & Poledne, R. (2018). Cardiovascular disease predictors and adipose tissue macrophage polarization: Is there a link? *European Journal of Preventive Cardiology*, 25(3), 328-334. doi:10.1177/2047487317743355
- Liu, T., Ai, Y., Ding, J., Li, B., Zeng, C., Zhang, X., Zhong, H., & Zhou, Z. (2022). Synthesis, crystal structure, DFT and anticancer study of dihydroartermisinin-paracetamol hybrids. *Journal of Molecular Structure*, 1250, 131758.
- Mirrasekhian, E., Nilsson, J.L., Shionoya, K., Blomgren, A., Zygmunt, P. M., Engblom, D., Högestätt, E. D., & Blomqvist, A. (2018). The antipyretic effect of paracetamol occurs independent of transient receptor potential ankyrin 1-mediated hypothermia and is associated with prostaglandin inhibition in the brain. *The FASEB Journal*, 32(10), 5751-5759.
- Ohtani, H., Ørskov, A. D., Helbo, A. S., Gillberg, L., Liu, M., Zhou, W., Ungerstedt, J., Hellström-Lindberg, E., Sun, W., Liang, G., et al. (2020). Activation of a subset of evolutionarily young transposable elements and innate immunity are linked to clinical responses to 5-azacytidine. *Cancer Research*, 80(12), 2441-2450.
- Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, 4(2), 89-96.
- Ralapanawa, U., Jayawickreme, K. P., Ekanayake, E. M. M., & Dissanayake, A. D. M. (2016). A study on paracetamol cardiotoxicity. *BMC Pharmacology and Toxicology*, 17, 1-8.
- Saliba, S. W., Marcotegui, A. R., Fortwängler, E., Ditrich, J., Perazzo, J. C., Muñoz, E., de Oliveira, A. C. P., & Fiebich, B. L. (2017). Paracetamol metabolite, prevents prostaglandin synthesis in activated microglia by inhibiting COX activity. *Journal of Neuroinflammation*, 14(1), 246.
- Santos, C. B., Lobato, C. C., Ota, S. S., Silva, R. C., Bittencourt, R. C., Freitas, J. J., Ferreira, E. F., Ferreira, M. B., Silva, R. C., De Lima, A. B., et al. (2023). Analgesic activity of 5-Acetamido-2-hydroxy benzoic acid derivatives and an in-vivo and in-silico analysis of their target interactions. *Pharmaceuticals*, 16(11), 1584.
- Shertzer, H. G., Schneider, S. N., Kendig, E. L., Clegg, D. J., D'Alessio, D. A., & Genter, M. B. (2008). Acetaminophen normalizes glucose homeostasis in mouse models for diabetes. *Biochemical Pharmacology*, 75(6), 1402-1410.
- Srivastava, R., Ali, M., & Nishad, U. (2020). Synthesis and biological activity of different aromatic compounds of benzimidazole derivatives. *Journal of Pharmaceutical Sciences and Research*, 12(4), 507-515.
- Stella, V. J. (2020). Prodrugs: My initial exploration and where it led. *Journal of Pharmaceutical Sciences*, 109(12), 3514-3523.
- Turones, L. C., Machado, L. S., Vaz, B. G., de Almeida R. Oliveira, G., da Silva Moreira, L. K., Almeida, D. D. S., Martins, A. N., Fajemiroye, J. O., Martins, J. L. R., Ghedini, P. C., et al. (2023). Anti-inflammatory and antinociceptive effects, and safety toxicological profile of a new paracetamol analog, LQFM291. *Inflammopharmacology*, 31(5), 2451-2465. doi:10.1007/s10787-023-01324-8