Journal of Mind and Medical Sciences

Volume 9 | Issue 1 Article 10

2022

Acetaminophen, a therapeutic or an extremely toxic remedy – a review

Genica Caragea
Military Medical Research Center Bucharest, ginacaragea@yahoo.com

Oana Avram
Carol Davila University of Medicine and Pharmacy, oana.avram@umfcd.ro

Andreea Pauna
Carol Davila University of Medicine and Pharmacy, andreea.pauna@umfcd.ro

Andreea Cristina Costea
Diaverum Clinic Constanta, acostea2021@gmail.com

Miruna Tudosie
Regina Maria Polyclinic Bucharest, mirunatudosie@yahoo.com

Journal of Mind and Medical Sciences

https://scholar.valpo.edu/jmms/
https://proscholar.org/jmms/

ISSN: 2392-7674

Acetaminophen, a therapeutic or an extremely toxic remedy – a review

Genica Caragea[1], Oana Avram[2,3], Andreea Pauna[2], Andreea Cristina Costea[4*], Miruna Tudosie[5]

[1]*Military Medical Research Center, Bucharest, Romania, 010919*
[2]*Carol Davila University of Medicine and Pharmacy, Department of Toxicology, Bucharest, Romania*
[3]*Emergency Clinic Hospital of Bucharest, Bucharest, Romania*
[4]*Diaverum Clinic, Department of Nephrology and Dialysis, Constanta, Romania*
[5]*Regina Maria Polyclinic, Department of ophthalmology, Bucharest, Romania*

ABSTRACT

When a new coronavirus appeared in the late 2019, identified as the cause of several cases of pneumonia in Wuhan, Paracetamol was initially reported to be the preferable antipyretic medication, choice which was detrimental to the use of other drugs. People have resorted to buying large stocks of Paracetamol and some have used it in large doses, regardless of the consequences. However, the Paracetamol (Acetaminophen) overdose remains the leading cause of death or transplantation due to acute liver failure in many parts of the world. This review aims at presenting the pharmacokinetics, the clinical signs, and the risk factors for systemic toxicity associated with Paracetamol overdose, as well as the current therapeutic approach. Paracetamol is primarily metabolized in the liver, by glucuronidation and sulfation. In case of a Paracetamol overdose, a large amount of NAPQI is conjugated with glutathione, and this process is due to a major depletion of glutathione, thus leading to hepatic necrosis, renal failure, and encephalopathy. The evaluation of serum acetaminophen levels by analytical methods is extremely useful both for the diagnosis and the therapy monitoring.

Category: Review

Received: December 12, 2021
Accepted: February 19, 2022
Published: April 10, 2022

Keywords:

acetaminophen, overdose, liver failure, analytical methods

***Corresponding author:**

Andreea Cristina Costea,

Diaverum Clinic, Department of Nephrology and Dialysis, Constanta, Romania, 900612

E-mail: acostea2021@gmail.com

Introduction

When a new coronavirus appeared in the late 2019, identified as the cause of several cases of pneumonia in Wuhan, doctors went on alert to understand the course of the disease and to identify the optimal treatment solutions [1]. The etiologic agent, called SARS-Cov2, is a newly discovered ARN virus in the Coronavirus family, generally responsible for benign respiratory infections, except for the causative agents of MERS (2012) and SARS (2002-2003) outbreaks [2,3]. Being a new pathogen and a new disease, the treatment options were limited, the only solution being to use drugs already in use, which had proven safe to use on humans and effective in treating other conditions and, thus, to improve the symptoms and to support vital functions. The WHO has issued Guidelines on how to care for patients with COVID-19, which is still temporary due to the need to adapt and change in order to keep up with the new emerging data.

The health crisis generated by COVID-19 is also seen in the increasing demand for Paracetamol, a drug included in the therapeutic protocol for mild cases. During this period, Paracetamol was indicated to be used, which was detrimental to the use of other drugs. People have resorted to buying large stocks of Paracetamol and some have used it in large doses, regardless of the consequences. The demand for Paracetamol increased by 20% in the first months after the outbreak. During the pandemic, the volume of requests for paracetamol increased by more than 110%. Thus, the indication of its use as an anti-inflammatory drug, the lack of a prescription, and the ease in procuring it, led, in some cases, to lethal consequences.

The Paracetamol (acetaminophen) overdose remains the leading cause of death or transplantation due to acute liver failure in many parts of the world.

Paracetamol was first made in 1877 and it still is one of the most commonly used medications for pain and fever [4].

To cite this article: Genica Caragea, Oana Avram, Andreea Pauna, Andreea Cristina Costea, Miruna Tudosie. Acetaminophen, a therapeutic or an extremely toxic remedy – a review. *J Mind Med Sci*. 2022;9(1):102-110. doi: 10.22543/7674.91.P102110

Acetaminophen is an analgesic, non-morphine compound, widely used worldwide, due to its pharmacotherapeutic characteristics and very good tolerance in therapeutic doses. Acetaminophen is an analgesic drug used alone or in combination with opioids for pain management, as well as an antipyretic agent [5]. Paracetamol is close to classical nonsteroidal anti-inflammatory drugs. Acetaminophen (Paracetamol, N-acetyl-p-amino-phenol, Tylenol) is used in its pure form in many trade preparations for oral use or in combination with other substances, such as codeine and propoxyphene, pseudoephedrine, chlorpheniramine, ibuprofen, tramadol, and thanks to the analgesic and antipyretic effects, they are widely used for mild to moderate pain and fever. Postoperative pain is one of the main factors of postoperative discomfort and worry in the first postoperative evening [6]. However, a careful evaluation of the associated conditions is important in order to prevent toxicity-related side-effects. The paracetamol/ibuprofen combination provides a further increase in potency and it is superior to any drug used alone [7,8]. The confusion about the dosing of this drug may be caused by the availability of different formulas, strengths, and dosage instructions for children of various ages [7-9] Figure 1.

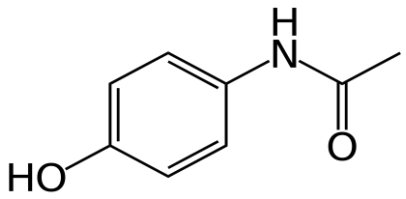

Figure 1. The chemical structure of Acetaminophen (PubChem)

Discussion

Pharmacokinetics and the mechanism of action

Acetaminophen is rapidly absorbed from the gastrointestinal (GI) tract and reaches the therapeutic levels within 30 minutes to 2 hours. Acetaminophen has an elimination half-life of 2 hours, but it can also last up to 17 hours in patients with hepatic dysfunction.

Mechanism of action

Paracetamol inhibits prostaglandin synthesis by reducing the active form of COX-1 and COX-2 enzymes. The paracetamol metabolite AM404 has been detected in the brains of animals and in the cerebrospinal fluid of humans after paracetamol administration [10,11].

The induction of fever is mediated by the release of pyrogenic cytokines, such as tumor necrosis factor α, interleukin 1, and interleukin 6 [12,13]. Honarmand et al. found that intravenous paracetamol significantly decreases the levels of Il-6 in patients in septic conditions in the ICU [14].

Paracetamol's bioavailability is dose-dependent: it increases from 63% for a 500-mg dose to 89% for a 1,000-mg dose [15]. The volume of distribution is about 0.9L/kg. About 10 to 20% of the drug is bound to red blood cells [16]. Acetaminophen appears to be widely distributed throughout most body tissues except for fat.

Paracetamol is metabolized primarily in the liver. The major route of metabolization consists in glucuronidation and sulfation. The products are then eliminated mainly in the urine. Additionally, 25–35% of paracetamol is converted into sulfate by means of the sulfation enzymes SULT1A1, SULT1A3, and SULT1E1 [17].

A second minor metabolic pathway (5-15%) of acetaminophen metabolization is oxidation by cytochrome P450 enzymes. The metabolite form that results is known as NAPQI (N-acetyl-p-benzoquinone imine) [17]. This metabolite is an N-hydroxylate derivative and it is responsible for all systemic damages caused by paracetamol. In normal conditions, this metabolite is detoxified by conjugation with glutathione. After conjugation with glutathione, this metabolite becomes non-toxic, because it is inactive.

The non-toxic conjugate APAP-GSH is taken up in the bile and further degraded into mercapturic and cysteine conjugates that are excreted in the urine.

GSH is a tripeptide that consists in the amino acids cysteine, glycine, and glutamate (Figure 2).

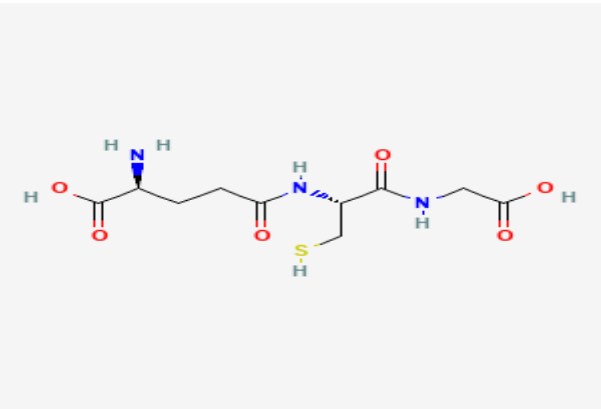

Figure 2. The structure of Glutathione (PubChem)

It possesses sulfhydryl donor groups and it can act as a powerful intracellular reducing agent and an antioxidant, whilst being converted into its oxidized form, glutathione disulfide (GSSG) [18]. The rate and the extent of GSH synthesis depend upon the local availability of cysteine, which is normally found in comparatively low concentrations in hepatocytes compared to other tissues [19].

Mechanisms of toxicity in paracetamol overdose

In paracetamol overdose, a large amount of NAPQI is conjugated with glutathione, and this process is due to a major depletion of glutathione. NAPQI binds to the mitochondria proteins of the liver cells causing oxidative stress and toxicity [17]. There is individual sensitivity to toxic doses of paracetamol. Some patients with toxic

concentrations of paracetamol in the plasma develop forms of fetal liver failure [20].

Another minor direction of metabolism is the deacetylation of 1–2% of paracetamol to form p-aminophenol. P-Aminophenol is then converted within the brain by fatty acid amide hydrolase into AM404, a compound that may be partially responsible for the analgesic action of paracetamol [10].

Harmless in low doses, acetaminophen has direct hepatotoxic potential when taken in an overdose and it can cause acute liver injury and death due to acute liver failure. Even in therapeutic doses, acetaminophen can cause transient serum aminotransferase elevations, especially in patients with existing comorbidities.

Acetaminophen may inhibit the nitric oxide (NO) pathway mediated by a variety of neurotransmitter receptors including N-methyl-D-aspartate (NMDA) and substance P, resulting in the elevation of the pain threshold. The antipyretic activity may result from the inhibition of prostaglandin synthesis and the release in the central nervous system (CNS) and the prostaglandin-mediated effects on the heat-regulating center in the anterior hypothalamus.

In acute intoxication, acetaminophen causes centrilobular liver necrosis. The necrosis of more than 60% of all hepatocytes causes fatal liver failure. Changes in secondary myocardial necrosis and subendocardial hemorrhage have also been reported.

Clinical signs of Paracetamol overdose

Acetaminophen toxicity is the second most common cause of liver transplantation worldwide. More than 60 million Americans use acetaminophen on a weekly basis, and many are unaware that it is contained in combined products [19-21].

The clinical course of acetaminophen toxicity may be divided into four stages [17]:

- Onset - during the first stage (30 min. to 24 hours), the patient may be asymptomatic or may have nausea, vomiting, anorexia, lethargy, diaphoresis.
- In the second stage - the latency stage (24 hours to 48 hours), the symptoms seem to be reduced almost entirely, but, paraclinically, the transaminases, bilirubin, and prothrombin time change.
- In the third stage (72 hours to 96 hours), liver dysfunction is significant, which might lead to renal failure, coagulopathies, metabolic acidosis, and encephalopathy. In this stage, renal tubular necrosis may occur, these features being known as the hepatorenal syndrome. Gastrointestinal (GI) symptoms reappear, and death is most common at this stage.
- The fourth stage (4 days to 3 weeks) is marked by recovery.

The diagnosis and management of Paracetamol intoxication

The diagnosis of Paracetamol intoxication is based on anamnestic elements, symptomatology, and paraclinical changes. Among the methods for identifying paracetamol, there are the colorimetric ones, or more precise methods, i.e. the chromatographic methods: Gas chromatography combined with mass spectrometry, HPLC. Biological samples must be withdrawn at least 4 hours after ingestion. The diagnosis of acetaminophen toxicity is based on the serum levels of the drug. Other important laboratory evaluations should include liver function tests and coagulation profiles.

In the therapeutic dose, acetaminophen is massively excreted in the urine as various conjugated compounds, for example as glucuronide-44-55% conjugate, 20-30% as sulfate, and 15-55% as a conjugated cysteine and mercapturic acid. Approximately 2% of the dose is eliminated unchanged. The overdose saturates the conjugate pathways and the glutathione reserve begins to deplete, leading to the formation of a highly active metabolite of acetaminophen, possibly an epoxide, a toxic intermediate that irreversibly combines with the constituents of the hepatocyte causing cell disruption [22,23].

Acetaminophen plasma concentrations in patients with an overdose may be between 30-300 mg/ L, and the half-life of the substance is considered to be a good indicator of hepatic impairment. For example, researchers looked at the changes in the serum levels of some microRNAs (miRNAs) after the exposure of mice to acetaminophen and their correlation with the severity of liver histopathological lesions modified in the early stages, even before the histopathological changes. It may be a biomarker of toxic liver damage, but miRNA-192 cannot be considered a sufficiently sensitive and specific biomarker for toxic liver damage [24,25].

The Laboratory of Analytical Toxicology plays an important role in the confirmation and the treatment of acetaminophen intoxication. The concentrations of acetaminophen in the serum or plasma depend on the time elapsed from ingestion to sample collection, on the conditions of collection and storage of the sample, but also the individual variations in the absorption, distribution, metabolism, and elimination of acetaminophen. In clinical toxicology, the chemical analysis is performed for diagnostic, therapeutic, and, over time, legal purposes. There are many methods for determining the serum level of acetaminophen, but they are often time-consuming, demanding, and require the use of expensive specialized tools.

The most used analytical methods are liquid and gas chromatographic methods, UV spectrophotometry methods, and immunochemical colorimetric methods.

Due to the possibility of separating nanograms of substance from an environment as complex as the biological product, chromatography has proved its true value, arousing special interest for toxicological analysis. Among the chromatographic methods, we mention thin layer chromatography, gas chromatography, high-performance liquid chromatography (HPLC), gas-chromatographic method combined with mass spectrometry, in order to identify the substances in the matrix obtained from biological samples [26].

In comparison with chromatographic techniques, electrochemical sensors can be directly applied to provide real-time sample information. Various electrochemical techniques, cyclic voltammetry, differential pulse voltammetry, and square wave voltammetry have been mostly employed in the determination of acetaminophen with high sensitivity. Immunochemical methods use an antibody specific for the acetaminophen molecule and a labeled form thereof.

The fast and semi-automatic immunochemical methods are EMIT (Enzyme Multiplied Immunoassay Technique) and FPIA (Fluorescence Polarization Immunoassay Technique). For example, the determination of serum acetaminophen by EMIT in Kinetic mode with a measurement time of at least 132 seconds at a wavelength of 340 nm is performed using a 6-point calibration curve using Emit® tox TM Syva concentration calibrators. 0, 10, 25, 50, 100, respectively 200 μg / ml (Figure 3).

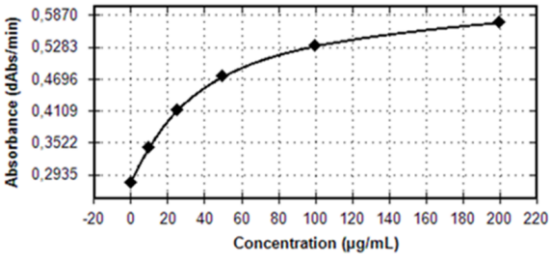

Figure 3. Calibration curve plotted with Emit® tox TMD acetaminophen calibrators/controls Syva® in the range 0-150 μg / ml on the Siemens Viva-ProE analyzer

In this way, the normal (therapeutic) concentrations of serum acetaminophen are within the range of 10-30 μg/ ml for healthy subjects [27].

Colorimetric techniques are commonly used in clinical laboratories, but they may interfere with acetaminophen metabolites, salicylates, phenacetin, or hemolyzed serum.

More than 30 years ago, Gupta RM et al. described a colorimetric method for the emergency determination of plasma acetaminophen. Acetaminophen was extracted into ethyl acetate at a physiological pH in order to remove salicylate, amino acids, and other polar compounds. The extract is treated with anhydrous sodium sulfate to remove traces of aqueous droplets containing protein or uric acid [28]. In 2001, Afshari and Liu described a rapid method for the determination of serum acetaminophen by means of the spectrophotometric method. Unconjugated free acetaminophen is separated from other endogenous interferences by its extraction into ethyl acetate and hydrolysis to p-aminophenol by heat and acid treatment. The latter compound is capable of undergoing an oxidative coupling reaction with sodium periodate-catalyzed p-xylene (2,5-dimethylphenol). The resulting indophenol derivative is measured spectrophotometrically at 635 nm. The proposed method has a linearity range from 25 to 600 μg / ml [29,30].

Chromatographic separation techniques have been commonly used for the determination of acetaminophen. LC-MS methods are more common than GC-MS methods because acetaminophen is a non-volatile compound and for the GC-MS analysis of acetaminophen, derivatization plays a key role. The modern chromatographic methods for acetaminophen which are currently used are MS and MS/MS detection and other detection methods, such as diode array detector (DAD) [30-32].

The development and validation of the high-performance liquid chromatography (HPLC) analysis using a Dionex Ultimate 3000 liquid chromatograph equipped with a multidimensional detector was established by de Ohriac et al [33]. After determining the optimum conditions of analysis (80/20 water/ acetonitrile mobile phase, flow rate 1.0 mL/ min, detection wavelength 245 nm), the method was validated with the following parameters: linearity of response function, linearity of results, limit (LD = 0.66 μg/ mL) and quantification limit (LQ = 2.00 μg/ mL) [34,35].

The mass spectrum of acetaminophen (MW = 151) is characterized by a baseline spectral line with m/ z 109 formed by hydrogen transfer from the methyl group of the acetyl fragment to ionized nitrogen, the mass spectral line m/ z 151, and a spectral line of m/ z 43 resulting from dissociation, with the formula C2H3O.

A GC-MS method for the identification of acetaminophen in urine was developed using a capillary gas chromatography-mass spectrometry with ion trap system Saturn 2000 GC 3800, with autosampler 3200 CX; the column used was FactorFour 30m x 0.25mm, ID DF=1.0 Varian/Chrompack. The operative parameters: Manifold temperature – 80^{o}C; Ion trap temperature – 170^{o}C; Acceleration voltage – 70 eV; The mass range 50–450 amu; The injector temperature – 300^{o}C; The interface GC-MS temperature – 260^{o}C; Carriere gas – He; The column flow 1.2 ml/min. The temperature program for the column was set to start at a 1400 C level for 2 minutes, followed by a temperature increase of 5^{0}C/ min to a 290^{0}C level for 13 minutes.

The confirmation of the identity of the compounds is based on the comparison between the mass spectrum and the ratio of the abundance of reference ions of each analyst identified in the sample and those of the standards, using

the mass spectrum library. NIST98 and PMW (Pfleger - Maurer – Weber) spectral libraries were used to identify the spectra obtained.

Following the analysis of the urine sample, the total chromatogram is shown in Figure 4, and thus the following compounds have been identified and are presented in Table 1: ibuprofen, acetaminophen, caffeine, chlorpheniramine.

Table 1. The analytical data on identified compounds

The identified compound	Molecular weight (atomic mass units)	Retention time (min)	CAS. No.
Ibuprofen	206	14,864	15687-27-1
Acetaminophen	151	18,783	103-90-2
Caffeine	194	19,550	58-08-2
Chlorphenamine	274	22,717	132-22-9

On the ion chromatogram total, the chromatographic peak belonging to the acetaminophen compound can be observed at 18.783 minutes, a compound identified using the NIST98 library (Figure 4).

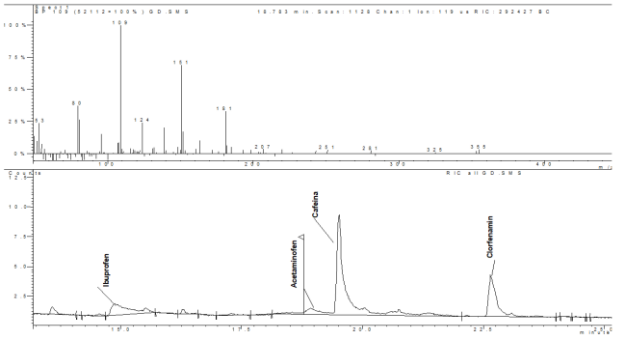

Figure 4. Total chromatogram ion and the mass spectrum obtained for Acetaminophen after the injection of 1µL of urinary extract into GC/ MS, with ion hatch in EI.

Another qualitative determination of acetaminophen and its major metabolite (3-methoxyparacetamol) from urine is presented using the gas chromatograph system combined with the mass spectrometer (GC-MS) quadrupole type, GC MSD QQQ 8890/7010 AGILENT with Agilent column HP25 5m2 Ultra, Um Splitless injection, Injected volume 1 ul, Injector temperature: 280°C, Carrier gas: He, with a total flow of 2.25 ml/ min; column flow: 1.1 ml/min, Detection mode: SCAN

In this case, the operative parameters for the mass spectrometer are: The source temperature = 2,300 C, the temperature on the first quadrupole = 1,500 C, the temperature on the second quadrupole = 1,500 C, the filament current = 100 microA, the type of ionization-electronics, the acceleration voltage = 70 eV.

MSD operates in Full SCAN mode and on analysis of the chromatogram obtained, ibuprofen (compound 1), paracetamol (compound 2), nicotine (compound 3), 3-methoxyparacetamol (compound 4), and caffeine (compound 5) were identified (Figure 5).

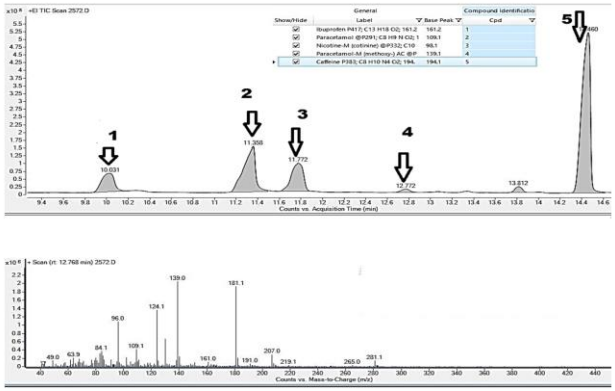

Figure 5. Total chromatogram ion and the mass spectrum obtained 3-methoxyparacetamol after the injection of 1µL of urinary extract into GC MSD QQQ 8890/7010 AGILENT

Gas chromatography with or without derivatization is the most specific technique. HPLC is sensitive and specific and it has the advantage of being used to measure the conjugated metabolites of acetaminophen in the blood and urine.

The determination of serum acetaminophen levels by TDM (Therapeutic Drug Monitoring) in the case of acute intoxications is important both for making the diagnosis of certainty, and especially for the correct application of the medical measures that are required mainly due to its high hepatotoxicity.

The therapeutic approach in suspected Paracetamol toxicity

Several factors were associated with an increased risk of Paracetamol toxicity, such as acute or chronic malnutrition, alcohol consumption. Diabetes mellitus and hyperglycemia were found to exacerbate acetaminophen-induced acute liver injury due to an increased level of oxidative stress and low-grade chronic inflammation [36-38]. Wide inter-individual variability was correlated with the numerous genes that may interfere with its pharmacokinetics and metabolization. Severe liver injury, along with the increasing expressions of NLRP3 and IL-1β, occurred in acetaminophen-treated mice liver or liver cell lines [39-41], while genetic deficiencies in NLRP3 inflammasome components (NLRP3, ASC, and caspase-1) were found to provide some degree of protection from acetaminophen-induced mice death and liver injury [42]. The toxicity of the drug in children below 12 years of age is still a controversial topic. However, there is evidence that the metabolization of the drug occurs predominately by sulfation if compared to adults, and no oxidation metabolism products were found in the children's plasma. The prenatal exposure to paracetamol was found to be associated with an increased risk of attention disorders and

ADHD, while these effects were not registered in postnatal exposure [43,44].

Phenobarbital-type enzyme inducers intensify the activity of oxidases with mixed microsomal functions, increasing the percentage of toxic metabolite and increasing the hepatotoxicity of paracetamol [45].

In order to reduce the systemic toxicity of Paracetamol and the functional recovery of the liver, Acetylcysteine is used as a specific antidote. Acetylcysteine is a prodrug of L-cysteine, which is a precursor of glutathione [46]. Acetylcysteine prevents GSH depletion and minimizes the hepatocyte injury caused by several different toxins. The potent electron donor properties of acetylcysteine and the consequent ability to lessen oxidative stress might be another independent mechanism that protects against acute liver injury [47].

Since N-acetylcysteine (NAC) is a precursor of glutathione, it increases the concentration of glutathione available for the conjugation of N-acetyl-p-benzoquinone imine (NAPQI) a toxic byproduct produced during the xenobiotic metabolism of Paracetamol [48]. The intravenous infusion regimen was developed to give the highest tolerated dose as quickly as possible because of the short-time window of therapeutic efficacy. NAC works through multiple routes. It prevents the binding of NAPQI to hepatic macromolecules, it acts as a substitute for glutathione, it is a precursor for sulfate, and it reduces NAPQI back to acetaminophen. The indications for NAC include serum levels that fall in the toxic range according to the Rumack-Matthew nomogram. The Rumack–Matthews nomogram or acetaminophen can be used to predict hepatotoxicity when the time of ingestion is known or a reference determination is made to allow the calculation of the Paracetamol half-life and the evaluation of the prognosis. It is a logarithmic graph starting not directly from ingestion, but from the 4 hour-post-ingestion after absorption is considered likely to be complete [49-51].

The NAC administration should still be attempted and it may improve survival even in delayed presentations, as it can act as an antioxidant that diminishes hepatic necrosis, decreases neutrophil infiltration, improves microcirculatory blood flow, and increases tissue oxygen delivery [52]. Acetylcysteine is extensively liver metabolized, CYP450 minimal, urine excretion is 22-30% with a half-life of 5.6 hours in adults and 11 hours in neonates. Hemodialysis can also be an effective treatment, especially with concurrent renal failure [52,53].

The overall prognosis of patients depends on the following criteria: creatinine levels higher than 3.4 mg/dL; arterial pH remaining lower than 7.3, despite the adequate fluid hydration; prothrombin time more than 1.8 times control or an INR of more than 6.5; the development of grade 3 or 4 encephalopathies [52-54].

Highlights

- ✓ Paracetamol is one of the most used over-the-counter medicines, but also a leading cause of toxic liver failure.
- ✓ The evaluation of serum acetaminophen levels by analytical methods is extremely useful both for the diagnosis and the therapeutic monitoring.

Conclusions

Paracetamol is one of the most used over-the-counter medicines, even after more than 100 years after its discovery, being a very efficient antipyretic and analgesic drug. In overdoses, it remains an important cause of morbidity, mortality and associated costs in healthcare worldwide. Understanding the metabolic pathways, the clinical signs, and the risk factors of systemic toxicity is important to prevent irreversible organ damage. The determination of serum acetaminophen levels by analytical methods is extremely useful both for diagnosis and the therapy monitoring.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

References

1. Costea DO, Enache FD, Baz R, Suceveanu AP, Suceveanu AI, Ardeleanu V, Mazilu L, Costea AC, Botea F, Voinea F. Confirmed child patient with covid-19 infection, opperated for associated surgical pathology – first pediatric case in Romania. *Rom Biotechnol Lett.* 2020;25(6):2107-2110. doi: 10.25083/rbl/25.6/2107.2110
2. Dascalu AM, Tudosie MS, Smarandache GC, Serban D. Impact of COVID-19 pandemic upon ophthalmological clinical practice. *Rom J Leg Med*. 2020;28(1):96-100. doi: 10.4323/rjlm.2020.96
3. Serban D, Socea B, Badiu CD, Tudor C, Balasescu SA, Dumitrescu D, Trotea AM, Spataru RI, Vancea G, Dascalu AM, Tanasescu C. Acute surgical abdomen during the COVID-19 pandemic: Clinical and therapeutic challenges. *Exp Ther Med*. 2021 May; 21(5):519. doi: 10.3892/etm.2021.9950
4. Prescott LF. Paracetamol: past, present, and future. *Am J Ther*. 2000 Mar;7(2):143-7.

5. Blondell RD, Azadfard M, Wisniewski AM. Pharmacologic therapy for acute pain. *Am Fam Physician*. 2013 Jun 1;87(11):766-72.
6. Șerban D, Brănescu CM, Smarandache GC, Tudor C, Tănăsescu C, Tudosie MS, Stana D, Costea DO, Dascălu AM, Spătaru RI. Safe surgery in day care centers: focus on preventing medical legal issues. *Rom J Leg Med*. 2021;29(1):60-64. doi: 10.4323/rjlm.2021.60
7. Bailey E, Worthington HV, van Wijk A, Yates JM, Coulthard P, Afzal Z. Ibuprofen and/or paracetamol (acetaminophen) for pain relief after surgical removal of lower wisdom teeth. *Cochrane Database Syst Rev*. 2013; (12):CD004624. doi: 10.1002/14651858.CD004624.pub2
8. Moore PA, Hersh EV. Combining ibuprofen and acetaminophen for acute pain management after third-molar extractions: translating clinical research to dental practice. *J Am Dent Assoc*. 2013 Aug;144(8):898-908. doi: 10.14219/jada.archive.2013.0207
9. Ekremoğlu M, Severcan C, Pasaoğlu ÖT , Şen B, Pasaoğlu H. An investigation of acute effects at various doses of malathion on glucose homeostasis and insulin resistance in rat liver, pancreas and serum. *J Mind Med Sci*. 2020;7(1):85-93. doi: 10.22543/7674.71.P8593
10. Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology*. 2013 Jun;21(3):201-32. doi: 10.1007/s10787-013-0172-x
11. Sharma CV, Long JH, Shah S, Rahman J, Perrett D, Ayoub SS, Mehta V. First evidence of the conversion of paracetamol to AM404 in human cerebrospinal fluid. *J Pain Res*. 2017 Nov 28;10:2703-2709. doi: 10.2147/JPR.S143500
12. Brănescu C, Serban D, Dascălu AM, Oprescu SM, Savlovschi C. Interleukin 6 and lipopolysaccharide binding protein - markers of inflammation in acute appendicitis. *Chirurgia (Bucur)*. 2013 Mar-Apr; 108(2):206-14.
13. Chiumello D, Gotti M, Vergani G. Paracetamol in fever in critically ill patients-an update. *J Crit Care*. 2017 Apr;38:245-252. doi: 10.1016/j.jcrc.2016.10.021
14. Honarmand H, Abdollahi M, Ahmadi A, Javadi MR, Khoshayand MR, Tabeefar H, Mousavi S, Mahmoudi L, Radfar M, Najafi A, Mojtahedzadeh M. Randomized trial of the effect of intravenous paracetamol on inflammatory biomarkers and outcome in febrile critically ill adults. *Daru*. 2012 Aug 28;20(1):12. doi: 10.1186/2008-2231-20-12
15. Liu DJ, Collaku A. Bioequivalence and Safety of Twice-Daily Sustained-Release Paracetamol (Acetaminophen) Compared With 3- and 4-Times-Daily Paracetamol: A Repeat-Dose, Crossover Pharmacokinetic Study in Healthy Volunteers. *Clin Pharmacol Drug Dev*. 2018 Jan;7(1):77-86. doi: 10.1002/cpdd.369
16. Bannwarth B, Péhourcq F. Bases pharmacologiques de l'emploi du paracétamol: aspects pharmacocinétiques et pharmacodynamiques [Pharmacologic basis for using paracetamol: pharmacokinetic and pharmacodynamic issues]. *Drugs*. 2003;63 Spec No 2:5-13.
17. McGill MR, Jaeschke H. Biomarkers of drug-induced liver injury: progress and utility in research, medicine, and regulation. *Expert Rev Mol Diagn*. 2018;18(9):797-807. doi: 10.1080/14737159.2018.1508998
18. Pereira CV, Nadanaciva S, Oliveira PJ, Will Y. The contribution of oxidative stress to drug-induced organ toxicity and its detection in vitro and in vivo. *Expert Opin Drug Metab Toxicol*. 2012 Feb;8(2):219-37. doi: 10.1517/17425255.2012.645536
19. Stipanuk MH, Dominy JE Jr, Lee JI, Coloso RM. Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. *J Nutr*. 2006;136(6 Suppl):1652S-1659S. doi: 10.1093/jn/136.6.1652S
20. Caparrotta TM, Antoine DJ, Dear JW. Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. *Eur J Clin Pharmacol*. 2018 Feb;74(2):147-160. doi: 10.1007/s00228-017-2356-6
21. Durmayüksel E, Çinar F, Guven BB, Aslan FE. Risk factors for the development of delirium in elderly patients undergoing orthopaedic surgery: A systematic review and meta-analysis. *J Clin Invest Surg*. 2021; 6(2):94-103. doi: 10.25083/2559.5555/6.2.3
22. Hinson JA, Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. *Handb Exp Pharmacol*. 2010;(196):369-405. doi: 10.1007/978-3-642-00663-0_12
23. Mazaleuskaya LL, Sangkuhl K, Thorn CF, FitzGerald GA, Altman RB, Klein TE. PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. *Pharmacogenet Genomics*. 2015 Aug;25(8):416-26. doi: 10.1097/FPC.0000000000000150
24. Yoon E, Babar A, Choudhary M, et al. Acetaminophen-Induced Hepatotoxicity: a Comprehensive Update. *J Clin Transl Hepatol*. 2016 Jun 28;4(2):131-42. doi: 10.14218/JCTH.2015.00052
25. Schiødt FV, Ott P, Christensen E, Bondesen S. The value of plasma acetaminophen half-life in antidote-treated acetaminophen overdosage. *Clin Pharmacol Ther*. 2002 Apr;71(4):221-5. doi: 10.1067/mcp.2002.121857
26. Grand-Guillaume Perrenoud A, Guillarme D, Boccard J, Veuthey JL, Barron D, Moco S. Ultra-high performance supercritical fluid chromatography coupled with quadrupole-time-of-flight mass spectrometry as a performing tool for bioactive analysis. *J Chromatogr A*. 2016 Jun 10;1450:101-11. doi: 10.1016/j.chroma.2016.04.053

27. Polson J, Wians FH Jr, Orsulak P, Fuller D, Murray NG, Koff JM, Khan AI, Balko JA, Hynan LS, Lee WM; Acute Liver Failure Study Group. False positive acetaminophen concentrations in patients with liver injury. *Clin Chim Acta*. 2008 May;391(1-2):24-30. doi: 10.1016/j.cca.2008.01.018
28. Gupta RN, Pickersgill R, Stefanec M. Colorimetric determination of acetaminophen. *Clin Biochem*. 1983 Aug;16(4):220-1. doi: 10.1016/s0009-9120(83)90028-0
29. Afshari JT, Liu TZ. Rapid spectrophotometric method for the quantitation of acetaminophen in serum. *Analytica Chimica Acta*. 2001;443(1):165–169. doi: 10.1016/S0003-2670(01)01198-9
30. Youssef SH, Mohamed D, Hegazy MAM, Badawey A. Analytical methods for the determination of paracetamol, pseudoephedrine and brompheniramine in Comtrex tablets. *BMC Chem*. 2019 Jul 2;13(1):78. doi: 10.1186/s13065-019-0595-6
31. Sukanya SD, Swamy BEK, Shashikumara JK, Sharma SC, Hariprasad SA. Poly (Orange CD) sensor for paracetamol in presence of folic acid and dopamine. *Sci Rep*. 2021 Nov 16;11(1):22332. doi: 10.1038/s41598-021-01311-5
32. Montaseri H, Forbes PBC. Analytical techniques for the determination of acetaminophen. *Trends Analyt Chem*. 2018; 108:122-134. doi: 10.1016/J.TRAC.2018.08.023
33. Ohriac Popa V, Cimpoesu D, Spac AF, Nedelea P, Lazureanu V, Suciu O, Popa TO, Butnaru E. The Determination of Paracetamol by HPLC Validation of the Method and Application on Serum Samples. *Rev Chim. (Bucharest).* 2018;69(3): 627-631. doi: 10.37358/RC.18.3.6163
34. Krauss M, Singer H, Hollender J. LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns. *Anal Bioanal Chem*. 2010 Jun;397(3):943-51. doi: 10.1007/s00216-010-3608-9
35. Boghitoiu D, Grama A, Pop T, Simionescu A, Ghita I, Ulmeanu EC, Nitescu V. The role of micro-RNAS as a diagnostic biomarker in the early prediction of acetaminophen-induced liver injury. *Farmacia*. 2021; 69(4):785-791. doi: 10.31925/farmacia.2021.4.21
36. Wang Q, Wei S, Zhou H, Shen G, Gan X, Zhou S, Qiu J, Shi C, Lu L. Hyperglycemia exacerbates acetaminophen-induced acute liver injury by promoting liver-resident macrophage proinflammatory response via AMPK/PI3K/AKT-mediated oxidative stress. *Cell Death Discov*. 2019 Jul 19;5:119. doi: 10.1038/s41420-019-0198-y
37. Dascalu AM, Stoian AP, Cherecheanu AP, Serban D, Costea DO, Tudosie MS, Stana D, Tanasescu D, Sabau AD, Gangura GA, Costea AC, Nicolae VA, Smarandache CG. Outcomes of Diabetic Retinopathy Post-Bariatric Surgery in Patients with Type 2 Diabetes Mellitus. *J Clin Med*. 2021 Aug 22;10(16):3736. doi: 10.3390/jcm10163736
38. Serban D, Papanas N, Dascalu AM, Stana D, Nicolae VA, Vancea G, Badiu CD, Tanasescu D, Tudor C, Balasescu SA, Pantea-Stoian A. Diabetic Retinopathy in Patients With Diabetic Foot Ulcer: A Systematic Review. *Int J Low Extrem Wounds*. 2021 Jun;20(2):98-103. doi: 10.1177/1534734620982237
39. Chen H, Wang Y, Jiao FZ, Yang F, Li X, Wang LW. Sinomenine Attenuates Acetaminophen-Induced Acute Liver Injury by Decreasing Oxidative Stress and Inflammatory Response via Regulating TGF-β/Smad Pathway in vitro and in vivo. *Drug Des Devel Ther*. 2020;14:2393-2403. doi: 10.2147/DDDT.S248823
40. Wei S, Ma W, Zhang B, Li W. NLRP3 Inflammasome: A Promising Therapeutic Target for Drug-Induced Toxicity. *Front Cell Dev Biol*. 2021 Apr 12;9:634607. doi: 10.3389/fcell.2021.634607
41. Suceveanu AI, Mazilu L, Katsiki N, Parepa I, Voinea F, Pantea-Stoian A, Rizzo M, Botea F, Herlea V, Serban D, Suceveanu AP. NLRP3 Inflammasome Biomarker-Could Be the New Tool for Improved Cardiometabolic Syndrome Outcome. *Metabolites*. 2020;10(11):448. doi: 10.3390/metabo10110448
42. Imaeda AB, Watanabe A, Sohail MA, Mahmood S, Mohamadnejad M, Sutterwala FS, Flavell RA, Mehal WZ. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. *J Clin Invest*. 2009;119(2):305-14. doi: 10.1172/JCI35958
43. Tudosie MS, Truta E, Davitoiu AM, Stanciulescu L, Jinescu G, Mitu AM, Forje M, Horhota L, Bojescu AA, Mares AM, Ionica M. The Impact of Copper in Children with Attention Deficit Hyperactivity Disorder. *Rev. Chim*. 2017;68(2):279-283. doi: 10.37358/RC.17.2.5436
44. Alemany S, Avella-García C, Liew Z, García-Esteban R, Inoue K, Cadman T, López-Vicente M, González L, Riaño Galán I, Andiarena A, Casas M, Margetaki K, Strandberg-Larsen K, Lawlor DA, El Marroun H, Tiemeier H, Iñiguez C, Tardón A, Santa-Marina L, Júlvez J, Porta D, Chatzi L, Sunyer J. Prenatal and postnatal exposure to acetaminophen in relation to autism spectrum and attention-deficit and hyperactivity symptoms in childhood: Meta-analysis in six European population-based cohorts. *Eur J Epidemiol*. 2021 Oct; 36(10):993-1004. doi: 10.1007/s10654-021-00754-4
45. Shao X, Wang P, Bao Y, Chen L, Zhong XB. Phenobarbital Increased Hepatic Toxicity of Acetaminophen due to Cytochrome P450 Induction in Young and Adult Mice. *The FASEB Journal*. 2019; 33(51):506.7-506.7.
46. Hołyńska-Iwan I, Wróblewski M, Olszewska-Słonina D, Tyrakowski T. The application of N-acetylcysteine

in optimization of specific pharmacological therapies. *Pol Merkur Lekarski*. 2017 Sep 29;43(255):140-144.
47. Acharya M, Lau-Cam CA. Comparison of the protective actions of N-acetylcysteine, hypotaurine and taurine against acetaminophen-induced hepatotoxicity in the rat. *J Biomed Sci*. 2010 Aug 24;17 Suppl 1(Suppl 1):S35. doi: 10.1186/1423-0127-17-S1-S35
48. Guicciardi ME, Malhi H, Mott JL, Gores GJ. Apoptosis and necrosis in the liver. *Compr Physiol*. 2013 Apr;3(2):977-1010. doi: 10.1002/cphy.c120020
49. Kale I. The predictive role of monocyte-lymphocyte ratio and platelet-lymphocyte ratio in postmenopausal osteoporosis. *J Clin Invest Surg*. 2021;6(2):141-147. doi: 10.25083/2559.5555/6.2.9
50. Rumack BH, Peterson RC, Koch GG, Amara IA. Acetaminophen overdose. 662 cases with evaluation of oral acetylcysteine treatment. *Arch Intern Med*. 1981; 141(3 Spec No):380-5. doi: 10.1001/archinte.141.3.380
51. Rumack BH. Acetaminophen hepatotoxicity: the first 35 years. *J Toxicol Clin Toxicol*. 2002;40(1):3-20. doi: 10.1081/clt-120002882
52. Ergin B, Guerci P, Zafrani L, Nocken F, Kandil A, Gurel-Gurevin E, Demirci-Tansel C, Ince C. Effects of N-acetylcysteine (NAC) supplementation in resuscitation fluids on renal microcirculatory oxygenation, inflammation, and function in a rat model of endotoxemia. *Intensive Care Med Exp*. 2016 Dec;4(1):29. doi: 10.1186/s40635-016-0106-1
53. Duygu Balpetek Külcü, Özge Cağcağ Yolcu. Consciousness level determination of red meat consumption of pregnant women, Giresun/Turkey province. *J Mind Med Sci.* 2020;7(1):79-84. doi: 10.22543/7674.71.P7984
54. Gosselin S, Juurlink DN, Kielstein JT, Ghannoum M, Lavergne V, Nolin TD, Hoffman RS; Extrip Workgroup. Extracorporeal treatment for acetaminophen poisoning: recommendations from the EXTRIP workgroup. *Clin Toxicol (Phila)*. 2014; 52(8):856-67. doi: 10.3109/15563650.2014.946994