CHAPTER - 1

INTRODUCTION

AND

LITERATURE REVIEW
GENERAL OVERVIEW

Paracetamol has been used as an analgesic for about 50 years and has relatively few side effects [1]. It is also known as acetaminophen in the United States. It was first used in medicine by Von Mering in 1893. Nevertheless, it has been gaining popularity since 1949, after it was recognized that the major active metabolite of both acetanilide and phenacetin were proved to be excessively toxic. Although paracetamol is a metabolite of both phenacetin and acetanilide but it does not share the renal or hematologic toxicity of its precursors. The molecular weight of paracetamol is 151.2 g mol⁻¹. It is a moderately water and lipid-soluble, like weak organic acid with a pKa of 9.5. Therefore, it is unionized over the physiological range of pH. Phenol has appreciable water soluble and apparently forms strong hydrogen bond. Preface of other radicals into the hydroxyl group of p-aminophenol and into the free amino group of aniline reduces toxicity without losing of antipyretic action [2].

![Molecular structure of paracetamol.](image)

It is classified as non-steroidal anti-inflammatory drugs (NSAIDs) in the textbooks of pharmacology [3, 4]. Like their archetype, most NSAIDs exhibit a triad of therapeutic activities, such as anti-inflammatory, antipyretic and analgesic. The NSAIDs also decrease prostaglandin (PG) biosynthesis by inhibiting the cyclooxygenase (COX) enzymes, either COX-1 or COX-2 or both. It has been accepted as their mode of action for more than 30 years [5, 6]. Paracetamol is relative to its antipyretic and analgesic activities and weak anti-inflammatory agent. It is also weak inhibitors of either COX-1 or COX-2 [7].
PHARMACOKINETICS PROPERTIES OF PARACETAMOL

Absorption

Paracetamol absorption appears to be negligible in the stomach, but very rapid in the small intestine. The mean absorption rates of paracetamol were similar in proximal and distal parts of small intestine [8]. In rats, absorption does occur slowly in the stomach and colon. It is more rapid in the small intestine, with 70% of the drug being absorbed within 30 minutes [9]. Absorption is followed by passive transport with first-order kinetics. The gastric emptying is the rate-limiting step in the absorption of paracetamol [10].

Paracetamol is rapidly absorbed in the gastrointestinal tract. However, it is incompletely available to the systemic circulation after oral administration, due to first-pass metabolism [11-13]. The concentration reaches a peak in 30 to 60 minutes and the plasma half-life is about 2 hours (hrs) after therapeutic doses [2]. The common therapeutic dose produces a plasma concentration of 5 to 20 µg mL\(^{-1}\). After 8 hrs, only a small amount of unchanged paracetamol is detectable in plasma [14].

Absorption of paracetamol seems to be predominantly dependent on the rate of gastric emptying, disease, or other condition which alters the rate of gastric emptying may influence the rate of paracetamol.

Distribution

Paracetamol distributes throughout most of the tissues and fluids. The tissue: plasma concentration ratio of paracetamol about unity in all tissues except fat and cerebrospinal fluid. With normal therapeutic dose, paracetamol is slightly bound to plasma proteins, only 20 to 50% may be bound at the concentrations encountered during acute intoxication [2]. Generally, the apparent volume distribution of paracetamol is about 1 L kg\(^{-1}\). The volume distribution is similar in healthy subjects, children and the elderly [15, 16].

Divoll et al. [17] showed that the intensity of distribution of paracetamol (corrected for weight) was larger in men (0.99 L kg\(^{-1}\)) than in
woman (0.86 L kg⁻¹) and declined with age in both sexes. The authors explained that the reduction in volume distribution of paracetamol in women and the elderly might be due to increased fat per kg body weight and incomplete distribution of nonlipophilic property of paracetamol into body fat.

**Metabolism**

Paracetamol is metabolized extensively in the liver and to a minor extent in the intestine [18, 19] (Figure 1.2). It is metabolized predominantly by glucuronide conjugation (approximately 60%) and sulfate conjugation (approximately 35%). Glucuronidation and sulfation require UDP (uridine diphospho)-glucuronyl transferase and sulfotransferase which are located in the endoplasmic reticulum and cytoplasmic compartments of the cell, respectively. In additional, both glucuronide and sulfate conjugations are capacity limited (saturable) processes [20, 21] that may be due to co-substrate depletion, i.e. Uridine diphospho gluconic acid (UDPGA) and 1'/phosphoadenosine-5'/phosphosulfate (PAPS) concentrations in the liver for glucuronidation and sulfation respectively. It appears that the formation of paracetamol glucuronide and sulfate conjugate in man becomes capacity limited upon administration of the 2.0 g dose of paracetamol [21]. In general, both conjugates are biologically inert metabolites and excreted mainly in the urine [22]. A small fraction of paracetamol is probably metabolized via oxidation by cytochrome P450. Two possible products of CYP-mediated paracetamol oxygenation are known, N-acetyl-p-benzoquinoneimine (NAPQI) and 3-hydroxyparacetamol, the latter compound being considered nontoxic [23]. The formation of NAPQI is dependent on some cytochromes P450 (CYP), identified in liver microsomes, in rats as CYP1A1, CYP1A2, and CYP2E1 [24]; in mice as CYP1A2 and CYP3A4 [25]; and in humans as CYP2E1, CYP1A2 and CYP3A4 [26, 27].

However, recent pharmacokinetics studies in human volunteers have demonstrated that the involvement of CYP1A2 and CYP3A4 in NAPQI formation in vivo is much less than that of CYP2E1. Omeprazole (CYP1A2
inducer) and rifampicin (CYP3A4 inducer) treatment has no effect on the formation of NAPQI from paracetamol. When, disulfiram (CYP2E1 inhibitor) treatment with paracetamol decreases the formation of NAPQI from paracetamol [28, 29].

![Pathways of paracetamol metabolism](image)

**Figure 1.2: Pathways of paracetamol metabolism [30].**

The toxic intermediate, N-acetyl-p-benzoquinoneimine (NAPQI), is produced through the N-oxidation of paracetamol to N-hydroxy paracetamol, followed by dehydration ([Figure 1.3]) [31].
However, many studies propose that N-hydroxy paracetamol is not formed as intermediate, instead of paracetamol undergoes one or two electron oxidation to quinoneimine reactive species (N-acetyl-p-benzo semiquinone (NAPSQI)) (Figure 1.4) [31].

Figure 1.4: Formation of NAPQI from paracetamol via NAPSQI.

The semiquinone radical, N-acetyl-p-benzo semiquinone (NAPSQI), intermediate of paracetamol might undergo a cyclic oxidation-reduction
process (Figure 1.4). It consists of the oxidation of the semiquinone to the quinoneimine by molecular oxygen, with generation of superoxide and followed by the reformation of the semiquinone by microsomal NADPH-cytochrome reductase (Figure 1.5). The hepatotoxicity of paracetamol might be due to production of the semiquinone free radical and active oxygen species, such as $\text{H}_2\text{O}_2$ and $\text{O}_2^\cdot$. Both the intermediate and active oxygen can bind and inactivate intracellular proteins [31].

NAPQI formed after the ingestion of a therapeutic dose of paracetamol is promptly detoxified by conjugation with glutathione. Glutathione is a sulfhydryl compound that plays an extremely important role in protecting liver, renal, and other organ damage from reactive metabolite of paracetamol (NAPQI). First, glutathione attacks NAPQI with the glutathione-s-transferase. Then, the glutamate and glycine portions are split off by gamma-glutamyl transpeptidase and cysteinyl glycine, respectively. The free cysteine-drug conjugate is finally acetylated by a cytoplasmic acetyltransferase to form a mercapturic acid conjugate. The cysteine and mercapturic acid conjugates are nontoxic metabolites and excreted in the urine [22].

**Elimination**

In young healthy subjects approximately 85-95% of therapeutic dose is excreted in urine within 24 hours, as unchanged paracetamol (4%), glucuronide (55%), sulphate (30%), cysteine (4%) and mercapturic acid conjugates (4%) [32, 33]. However, newborn and children aged 3 to 10 years excreted significantly less glucuronide and more sulfate conjugate than children aged 12 years and adults. In rats, sulfate conjugate and mercapturic acid are primarily excreted at lower dose of the drug whereas at higher dose, glucuronide conjugate is more dominant.

Other minor metabolites have been described, each accounting for 1% or less of a therapeutic dose. These include sulphate and glucuronide conjugates of 3-methoxy-paracetamol, 3-hydroxy-paracetamol and 3-methyl-thioparacetamol [34, 35]. As a moderate lipid-soluble, weak organic
acid, paracetamol undergoes considerable filtration with subsequent extensive tubular reabsorption. However, the excretion of paracetamol is independent of urinary pH, but appears to be weakly correlated with urine flow rate \([33, 36]\). The highly polar sulphate and glucuronide conjugates of paracetamol are apparently actively secreted by the tubules. It is indicated by their respective renal clearance rates of approximately 170 and 130 mL min\(^{-1}\). There is no correlation with urine flow or pH. The renal clearance of the sulphate conjugates of paracetamol is concentration dependent \([36]\).

**ADVERSE EFFECT OF PARACETAMOL**

Paracetamol is virtually free of any significant adverse effects. Skin rash and other allergic reactions take place occasionally. The rash is usually erythematous or urticarial, but sometimes it is more severe and may be accompanied by drug fever and mucosal lesions \([37]\). Patients who show hypersensitivity reactions to the salicylates only rarely exhibit sensitivity to paracetamol and related drugs. There may be minor alterations in leukocyte count, but these are generally transient \([37]\). In a few isolated cases, the use of paracetamol has been associated with agranulocytosis, neutropenia, thrombocytopenia, and pancytopenia \([2]\). Paracetamol causes methaemoglobinemia and oxidative hemolysis in dogs, pigs and cats, but not normally in humans, even after over dosage \([38]\). In chronic toxicity studies, paracetamol is less potential for nephrotoxicity than aspirin and other non-steroidal anti-inflammatory analgesics \([36]\). The strain-dependent cataract formation and other ocular abnormalities have been described in induced mice \([39]\). In one study; paracetamol produced a high incidence of liver cell tumors in 1F mice \([40]\). High doses of paracetamol given chronically to animals may cause testicular atrophy and inhibition of spermatogenesis \([41]\).

**HEPATOTOXICITY**

Paracetamol is induced hepatotoxicity by the formation of NAPQI and a metabolite formed by cytochrome P450. The quantitatively most significant
of these is CYP2E1 [26]. A number of recent reports indicate that massive overdose of paracetamol can produce a fulminate acute centrilobular hepatic necrosis in humans [42] and in animals [43]. There are considerable species differences in susceptibility.

The acute hepatotoxic doses in hamsters, mice, and rats are about 150 mg kg$^{-1}$, 300 mg kg$^{-1}$ and 3,000 mg kg$^{-1}$, respectively. In adults, hepatotoxicity may occur after ingestion of a single dose of 10 to 15 g (150 to 250 mg kg$^{-1}$) of paracetamol [14, 44-46]. The doses of 20 to 25 g or more are potentially fatal and death caused by severe hepatotoxicity with centrilobular necrosis, sometimes associated with acute renal tubular necrosis. Symptoms occur in the initial 24 hours and may persist for a week or more. Clinical indication of hepatic damage becomes evident within 2 to 4 days of ingestion of toxic doses.

Initially, plasma transaminases are elevated and the concentration of bilirubin in plasma may be increased. In addition, the prothrombin time is prolonged. Perhaps 10% of poisoned patients, who do not receive specific treatment, develop severe liver damage of these and also 10 to 20% eventually die due to hepatic failure. Acute renal failure also happens in some patients.

Severe liver damage occurs in 90% of patients with plasma concentrations of paracetamol greater than 300 µg mL$^{-1}$ at 4 hrs or 45 µg mL$^{-1}$ at 15 hrs after the ingestion of the drug [2, 47]. Minimal hepatic damage can be expected, when the drug concentration is less than 120 µg mL$^{-1}$ at 4 hrs or 30 µg mL$^{-1}$ at 12 hrs after ingestion [2]. The potential severity of hepatic necrosis can also be predicted from the half-life of paracetamol observed in the patient. The half-life values are greater than 4 hrs imply that necrosis will occur, while values greater than 12 hrs suggest that hepatic coma is likely [48, 49]. In patients with severe liver damage, it may be a progressive increase in the plasma half-life. Prolongation of the plasma paracetamol half-life is associated with a marked increase in the ratio of the plasma concentrations of unchanged to conjugated drug. The patients with fatal hepatic necrosis due to the conjugation of paracetamol may virtually cease.
Nephrotoxicity

Hepatotoxicity is the most remarkable feature of paracetamol overdose [50]. Renal effects of paracetamol overdose are less commonly seen than hepatic effects. However, renal impairment may be more common than previously recognized. The overall incidence of acute renal failure in patients with paracetamol poisoning is less than 2% [51], and acute renal failure occurs in 10 to 40% of patients with severe hepatic necrosis [52].

Previously, it was believed that paracetamol-induced kidney failure occurred as a result of severe hepatic failure [53, 54]. In the presence of severe hepatotoxicity that precludes further hepatic metabolism of the parent paracetamol, there may be ‘spill over’ of paracetamol to the kidney where it will be metabolized [55]. Nephrotoxicity then results when there is insufficient glutathione in the renal parenchyma. This was initially interpreted as a hepatorenal syndrome [56], where extreme intrarenal arterial and arteriolar vasoconstriction would be observed [57].

When paracetamol is metabolized in both the liver and kidney, nephrotoxicity may occur independently of hepatotoxicity depending on the balance of metabolism and the glutathione stores within the kidney [55]. Studies in the mouse suggest that biotransformation of paracetamol in the kidney, a reactive electrophile contributes to covalent binding and subsequent induce nephrotoxicity [58]. Mitchell et al demonstrated that certain strains of rats that have high concentrations of microsomal cytochrome P450 in their kidneys, developed acute tubular necrosis after a single, nonlethal dose of paracetamol [59]. Paracetamol has given in increasing doses to male Fischer rats depleted glutathione stores in the liver and kidneys; large amounts of oxidative radiolabelled metabolite bound to hepatic and kidney protein then led to a dose dependent acute hepatic and renal necrosis [60]. The incidence and severity of paracetamol-induced liver and renal necrosis decreased when the rats were pre-treated with cobalt chloride, an inhibitor of cytochrome P450.
LITERATURE REVIEW

Paracetamol (N-acetyl p-aminophenol) is widely used as an analgesic [61] and antipyretic drug [62]. Paracetamol is classified as a member of the non-steroidal anti-inflammatory drug (NSAIDs). Paracetamol and its derivatives are of great significance due to their important roles in pharmacological systems. The previous literature review also suggested that paracetamol is a very important compound which has been found to maintain and improve significant pharmacological activities. It is also important moiety in the creation of novel medical materials or derivatives. Adverse pharmacological activities of paracetamol and its derivatives has been attributed such as, antioxidant [63], anti-inflammatory [64-66], analgesic [65-67] and immunomodulatory [68] activities. Paracetamol is a very important pharmacological moiety and it produces a wide range of derivatives during the reaction with different organic moieties. 2-Oxazetidine and 5-Oximidazolidine derivatives of paracetamol show antimicrobial activity [69, 70]. Dicarboxylic acid bis(4-acetylamino phenyl) ester and (4-acetylamino phenoxo) acetic acid have been shown analgesic, antipyretic and anti-inflammatory [71], and Metacetamol derivatives show antibacterial and anthelmintic activity [72]. Inspite of the numbers of reports on the derivatization of the basic structure of paracetamol available in the literature, efforts are continuing to find to enhance the pharmacological activity of basic paracetamol for combining and diverse applications.

IMPORTANT SUBSTITUENTS

The sulphonamide, diclofenac sodium and norfloxacin remain of great interest due to the wide applications in the pharmaceutical arena.

Sulphanilamide or 4-amino phenyl sulphonamide:

![Molecular structure of sulphanilamide](image)

Figure 1.6: Molecular structure of sulphanilamide.
For many years, the sulfonamides have been widely studied for their chemotherapeutic activity. Sulphonamide is 4-aminobenzenesulphonamide and widely used as bacteriostatic agents [73-75], antifungal [76] carbonic anhydrase inhibitor [74], anticancer [77], anti-inflammatory [78], anti-HIV [79], COX-2 inhibitor [80], selective 5-HT receptor antagonist [81], antitubercular [82], antimalarial and antileprotic agents [83, 84]. Sulphanilamide derivatives have exposed significant biological activities such as, antimicrobial [85], anticonvulsant [86], analgesic [86, 87], anti-inflammatory [88] and antiviral [89] activities.

**Diclofenac Sodium or Sodium 2-[[[(2, 6-dichlorophenyl) -amino] phenyl] acetate):**

![Molecular structure of diclofenac sodium.](image)

Diclofenac sodium has been accepted a non-steroidal anti-inflammatory drug [90]. However, it has been suggested that, in addition to the inhibition of cyclooxygenase. Diclofenac sodium apparently possesses an additional mechanism of analgesic action [91]. Several reports of diclofenac sodium proved as an antimicrobial agent [92, 93]. Diclofenac sodium derivatives have been presented some potential pharmacological activities such as, antimycobacterial [94], antifungal [95], anti-inflammatory [96], analgesic and antipyretic.
Norfloxacin or 1-Ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carboxylic acid:

![Molecular structure of norfloxacin](image)

**Figure 1.8: Molecular structure of norfloxacin.**

Norfloxacin is chemically fluoroquinolone, 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid. Norfloxacin is a very important family of antibacterial agents [97, 98], it mainly contains quinolone moiety. Norfloxacin is a chemically active substitute and its various derivatives have exposed antibacterial [99-101], antituberculosis [102] and anti-HIV [103] activities.

This gave a great impetus to the search for potential pharmacologically active drugs carrying sulfonamide, diclofenac sodium and norfloxacin substituents.

**SPECTROPHOTOMETRIC METHOD OF PARACETAMOL**

Several analytical methods have been reported for the measurement of paracetamol, such as flow injection method [104], liquid chromatography [105], titrimetry [106], capillary electrophoresis [107], chemiluminescence [108], electrochemical techniques [109] and various spectrophotometric methods which are mainly based on nitration [110, 111], oxidation [112] and hydrolysis to p-aminophenol followed by diazotization and phenolic coupling [113, 114]. Moreover, HPLC techniques [115], electrochemical method [116], spectrofluorimetry [117] and, other than there are very few spectrophotometric methods [118-120] are known for determination of paracetamol in biological fluids. Therefore, an estimation of drug in various *in-vitro* and *in-vivo* pharmacological parameters like, pharmacokinetics,
pharmacodynamics and bioequivalence are needed to develop various analytical techniques.

**IMPORTANCE OF PHARMACOKINETICS STUDIES**

Synthesis of derivatives and examine various activities are not enough in the medicinal chemistry, but *in vivo* pharmacokinetic (PK) studies guides the medicinal chemists to optimize the chemical structure of compounds. The significant improvement was due to a change in drug discovery strategies, as a medicinal chemist began assessing PK properties of new chemical entities at a very early stage of drug discovery [121, 122]. *In vivo* animal PK information also assists pharmacologists to design effectively in vivo efficacy studies and accurately interpret pharmacodynamic (PD) observation. In recent years, the throughput of the drug discovery process has improved because of the implementation of high-throughput in vitro ADME (Absorption, Distribution, Metabolism, and Elimination) assay. ADME assay means examine the various pharmacological parameters such as absorption, distribution, metabolism and elimination. Hundreds of compounds can be screened in vitro per week, providing scientist with a wealth of data [123]. By contrast, *in vivo* PK studies are still conducted in a traditional low throughput manner in most pharmaceutical companies. Therefore, there is a need to bring in vivo PK studies into a higher throughput arena. Thus, PK study is very important for newly synthesize derivatives, that can be helpful in the clinical setting.
AIM AND OBJECTIVE OF PRESENT WORK

➢ To synthesize paracetamol derivatives through condensation reaction with various pharmacological active moieties such as, paracetamol, sulphanilamide, diclofenac sodium and norfloxacin.

➢ To characterize these synthesized paracetamol derivatives for structure elucidation by Elemental analysis, UV, FT-IR, $^1$H NMR and LC-MS studies.

➢ To examine the physical parameters such % yield, solubility, melting points, thin layer chromatography, pH and pKa studies.

➢ To develop a new spectrophotometric method for synthesized paracetamol derivatives and also apply for biological fluids.

➢ To evaluate the antimicrobial activity of paracetamol derivatives against different bacterial and fungal strains.

➢ To examine the acute toxicity study and pharmacological evaluation such as, analgesic, antipyretic, anti-inflammatory activities and pharmacokinetics study using blood and urine samples of rats.
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