A Review of the Biochemical, Hematological and Histological Modulations in Acetaminophen Induced Hepatotoxicity and the Potential of *Urtica Dioica* in the Regeneration of the Liver

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Abstract

Acetaminophen is a common antipyretic/analgesic drug available as an over the counter prescription. It is an acetylilide and phenacetin derivatives. Extensive studies on the safety of acetaminophen have been performed and evidence of hepatotoxicity remains to be established. It has been used in the management of fever and pain. In addition, it has been compounded with many other drugs raising concerns over the safety and efficacy of the use of acetaminophen. The toxicity effect of acetaminophen have also been sustained in all routes of administration; intravenous, intramuscular, rectal and oral with similar biochemical, hematological and histological profiles. The liver is the main organ responsible for metabolic biotransformation of acetaminophen. The hematological, biochemical and pathological effect of the acetaminophen hepatotoxicity will provide a better understanding of the mechanism of action in acetaminophen toxicity. This study therefore reviews previous studies assessing hepatotoxicity by examining the biochemical, hematological and histological findings as determined after acetaminophen administration. The potential for *Urtica dioica* in protecting the liver and its ability in regenerating the hematological, liver enzymes and tissues has been demonstrated in many other studies as shown in this study. Hence, there is great potential for *Urtica dioica* in the protection of the liver against acetaminophen induced hepatotoxicity. Statistical analysis is also very important for the interpretation of toxicity data. This study has also reviewed the relevant statistical methods as they have been used in various toxicological studies.

Keywords: Acetaminophen (APAP); Hepatotoxicity; N-acetyl-p-benzoquinoneimine; N-acetyl-p-aminophenol; Glutathione depletion; *Urtica dioica*; Hematoprotection; Hepatoprotection

Introduction

Acetaminophen belongs to the para-aminophenol group of compounds with acetanilide as the parent member. Acetaminophen is the major active metabolite of acetanilide and phenacetin. Acetanilide was introduced in medicine in 1866 for its antipyretic action but it proved to be excessively toxic. While phenacetin was introduced in 1887 but was prohibited for use in 1960 with the finding that it promoted nephrotoxicity among the chronic uses of the drug. Acetaminophen was introduced by Von Merring in 1893 as a potential drug working as an analgesic. However, its effectiveness as a therapeutic drug was only realized in the 1960. In therapeutic doses acetaminophen is very safe and it is currently used as an over the counter and a prescription drug for the treatment of pain and fever [1]. Acetaminophen use has increased with time leading to use as a combination in many other drugs as well.

Physical and Chemical Properties

Acetaminophen occurs as a white, odorless crystalline powder with a bitter taste when taken orally. It is nonflammable, with a melting point of between 169-172°C and a specific gravity of 1.293. The compound is miscible with water, methanol and ethanol and is prepared at a pH of about 5.5-6.5 [2]. Chemically, it is composed of a benzene ring and is referred to as N- (4 hydroxyphenyl) acetamide. It is formed through the interaction of p-aminophenol and an aqueous solution of acetic anhydride. Acetaminophen is also known as paracetamol in different jurisdictions. It has been used in other activities including: an intermediate in pharmaceutical production of penicillin; stabilizer for hydrogen peroxide; and as a component in the production of the photographic chemicals.

Acetaminophen is prepared as a tablet, capsule, liquid suspension, intravenous, intramuscular and effervescent form. The preparations are prepared in the form of common adult doses of between 500 mg to 1000 mg [2]. Acetaminophen has been described as safe for children, infants and adults at the recommended doses. In some cases, it is prepared as a combination with other formulations such as opioid codeine, dihydrocodeine, oxycodone and hydrocordone [2]. The root of administration of acetaminophen includes intravenous, intramuscular, rectal and oral. It is advisable that a minimum dose of 325 mg - 650 mg/kg be administered every 4-6 hours. FDA now recommends a maximum dose of acetaminophen at 325 mg/kg per tablet and any combination of prescriptions containing more than 325 mg of acetaminophen is banned from use since they cause liver damage [3,4].

Absorption and Distribution

After acetaminophen is administered orally, it is quickly absorbed by the GI tract and more specifically the small intestine. It is understood that the absorption occurs through passive diffusion as a form of transport. The bioavailability of acetaminophen is about 85% to 95%. Maximum plasma concentration of acetaminophen occurs...
within the first 10-90 minutes after ingestion and the plasma concentrations range between 8-32 µg/ml, at about 6 hours, the plasma concentration ranges from 1-4 µg/ml [5].

Acetaminophen is widely distributed in most of the body fluids except fat. It has been estimated that the apparent volume of distribution is 0.95 L/kg [5]. It is also estimated that about 10-25% of acetaminophen is plasma proteins bound. Binding is however increased in the incidence of overdose [6]. The sulfate and glucoronide metabolites of acetaminophen are not bound by plasma proteins even at their high concentrations [7]. As a result of low molecular weight and affinity to plasma proteins, it is able to cross over the blood brain barrier. Moreau et al. [8] established that peak concentration in cerebrospinal fluid is achieved in 2-3 hours. It has also been demonstrated that acetaminophen can cross the placental barrier at therapeutic doses into the fetus blood circulation 30 minutes after administration [9]. It has also been established that acetaminophen does not present a risk to nursing infants due to very low levels of acetaminophen detected in maternal milk at 0.1-1.85% even at peak plasma concentration levels [10].

Metabolism and Excretion

Extensive studies have been conducted on the metabolism of acetaminophen in the body and the pathway is well known [11]. The liver plays a significant role in the metabolism of acetaminophen. Three pathways have been noted as significant in the metabolism of acetaminophen [12]. It has been shown that glucuronidation accounts for about 55-60% of the metabolism of acetaminophen. Moreover, sulfation which is a conjugation reaction accounts for about 20-30% of the metabolic reactions of acetaminophen. Finally, N-hydroxylation, dehydration and conjugation of glutathione reactions account for less than 15% of the key metabolic steps involved in the metabolism of acetaminophen. The major enzyme involved in metabolism of acetaminophen is referred to as the hepatic cytochrome P 450 and it is understood on the basis of two schools of thoughts. First, is described as the glutathione depletion theory. This theory proposes that the first metabolic step of acetaminophen in the liver is by conversion to the reactive intermediate molecule N-acetyl-p-benzoquinoneimine (NAPQI) by the enzyme cytochrome P-450 [11]. An excess of the NAPQI is believed to react with glutathione leading to the formation of the glutathione conjugates. It has been suggested that more than 90% of the glutathione is depleted following acetaminophen toxicity. This is followed by oxidative stress which leads to the development of superoxide that promotes the formation of hydrogen peroxidase and creating peroxidase reaction which eventually promotes death of hepatocytes cells [19, 20]. The second theory is known as the macromolecular inhibition theory. It postulates that covalent binding takes place with the formation of NAPQI leading to the formation of the macromolecules that enables the inhibition of their function, eventually, cell death is said to occur [21-23].

Nelson [24] has postulated that mitochondrial proteins could be primary cellular targets by acetaminophen leading to the loss of activity of energy production in the cells. This study has been justified with findings that acetaminophen leads to the destruction of ATPase activity in the plasma membrane [23]. Other proteins adducts have also been identified in studies done on mice after administration of acetaminophen [23]. The levels of adduct in serum has been found to correlate with hepatic transaminase values in adults with acetaminophen-related liver failure [25]. These patients had been previously tested for other known causes of acute liver failure. In addition, adducts were recently shown to persist in serum for at least 12 days after severe acetaminophen overdose in adults [19].

Management of Acetaminophen Toxicity

Treatment using N-acetyl cysteine

Management of oral and intravenous acetaminophen overdose are the same. N-acetyl cysteine is considered the main antidote for the management of acetaminophen poisoning and it is co-administered with zinc and selenium. N-acetyl cysteine is theorized to work through a number of protective mechanisms [11]. Since NAC is a precursor of glutathione, it increases the concentration of glutathione available for the conjugation of NAPQI. N-acetyl cysteine also enhances sulfate conjugation of metabolized N-acetyl-p-aminophenol (APAP). In addition, it promotes the anti-inflammatory and antioxidant pathways and is associated with positive inotropic effects [18]. In addition, NAC has been shown to increase the local nitric oxide concentrations. This has been associated with the increased microcirculatory blood flow, promoting the delivery of local oxygen to peripheral tissues. These effects have been associated with a decrease in morbidity and mortality, even when NAC is administered in the setting of established hepatotoxicity [26].

N-acetyl cysteine is effective maximally for hepatoprotection only when used in the duration lasting 8 hours after acute acetaminophen ingestion. It is however indicated that NAC can be administered any
Alternative therapies

Several studies have shown the protective effect on acetaminophen toxicity through the targeting of the various pathways that lead to inhibition of NAPQI. For instance, Pretreatment of acute toxicity has also been managed with piperonyl butoxide [28]. In some instances, cobaltous chloride has also been used as a substitute for piperonyl butoxide. They have been indicated to protect against acetaminophen-induced hepatotoxicity in animals models [28]. Cimetidine is a drug that has been used in the management of patients with ulcers. Similarly, cimetidine has been shown to protect against hepatotoxicity of acetaminophen in animals. This has been achieved by inhibiting the metabolic biotransformation and activation of acetaminophen [29]. However, no studies have been done on human models to assess its role in hepatoprotection [30]. Parallel exposure of acetaminophen and ethanol appears to reduce activation of acetaminophen to reactive metabolites in rats [31]. On the other hand, the use of calcium channel blockers has also been demonstrated to reduce the development of liver necrosis after acetaminophen poisoning through the use of agents as nifedipine [32,33]. Other agents that have been tested include: inhibitors of phospholipase A2, cyclooxygenase and thromboxane synthetase [34]. 0.75% butylated hydroxyanisol has been shown to increase the concentration of reduced glutathione [35]. Finally, studies have also been done on the use of antioxidants and inhibitors of lipid peroxidation, the use of diethyldithiocarbamate and anisyldithiolthione, have been shown to promote acetaminophen induced hepatoprotection [36, 37].

Effects of Acetaminophen on Biochemical Parameters

Effects on hematological values

Oyediji et al. [38] showed that administration of a low dose of acetaminophen (7.5 mg/kg BW) caused non-significant (p>0.05) changes in hematological profiles: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), total white blood cells (TWBC), and platelet. The differential counts also had non-significant values relative to their controls. In a separate study Payasi et al. [39] reported that there were no significant hematological changes in the plasma levels of total RBCs, platelets, TWBCs, erythrocyte sedimentation rate (ESR) and Hb (p>0.05) in Winster rats following acetaminophen poisoning. Hematological findings using leaves of *Gnetum africanum* on acetaminophen-induced hepatotoxicity in rats showed significant changes in PCV, TWBC, neutrophils and Lymphocytes using 3 g/kg body weight of acetaminophen [40]. Similar studies using *Urtica dioica* are missing.

In another study which adopted the repeated model in acetaminophen administration for doses ranging between 100 mg/kg body weight to 500 mg/kg body weight, it was shown that acetaminophen induced damage to the liver promotes varying degrees of hematological parameters suggesting liver damage following changes in of Packed cell volume, Hb total leucocyte count (TLC), MCH, MCHC and MCV suggesting interference with the hematological profile at the different doses [41].

Effects on liver enzymes

A study on the effect of low doses of acetaminophen (16-66 mg/kg) was performed by Payasi et al. [39]. Findings showed insignificant changes in blood urea nitrogen, AST, ALT, ALP, glucose, bilirubin and creatinine levels with reference to their controls. However, in an earlier study a dose of 400 mg/kg body weight was shown to significantly elevate the levels of ALT starting 2 hours to 6 hours following acetaminophen administration [16]. Another study using 300 mg/kg, 400 mg/kg and 500 mg/kg of acetaminophen showed significant elevation in activity of ALT values suggesting damage to the liver [15]. Other studies have also shown that induced damage to the liver is associated with changes in liver enzyme activities in animals study models [44,45]. Other enzymes assessed include glutathione (GSH), superoxide dismutase (SOD), and Malonyl dialdehyde (MDA) [38].

Acetaminophen and liver necrosis

Histological studies on liver tissues following 7.5 mg/kg body weight administration of acetaminophen did not present any visible lesions on the liver tissues; they were similar to the control when observed [38]. Likewise at a dose of 16-66 mg/kg of acetaminophen administration, it has been shown that the liver tissues did not show any statistical significant differences in the histopathological change in
the liver of both female and male albino rats suggesting the absence of necrosis at this dose [39]. It has also been established that acetaminophen overdoses can cause liver injury and even failure in both animals and humans. Cell death may occur as a result of apoptosis and necrosis. In a study using 300 mg/kg of acetaminophen for induction of hepatotoxicity, it was shown that apoptotic cell death was present at 2 hours after acetaminophen administration. The apoptotic cell death increased significantly for about 6.3 cells out of 10 at about 6 hours after acetaminophen administration. The number of necrotic cells correlated with the increase in the activity of ALT in the cells [15]. Higher doses of acetaminophen have also been determined to promote necrosis in the same way. The study concluded that oncotic necrosis is the main mechanism of liver cell death after acetaminophen overdose in vivo [15].

The mode of cell death in acetaminophen induced hepatotoxicity was not influenced by the doses of acetaminophen following administration of 300 mg/kg, 400 mg/kg and 500 mg/kg. Histological assessment of the hepatic tissues showed severe liver injuries when killed at 6 hours. There was also significantly elevated activity of ALT values corresponding to the necrotic damage to the liver tissues. However, the number of apoptotic cells declined as the dose increased at the 3 doses of 300 mg/kg, 400 mg/kg and 500 mg/kg [15]. Another study also showed significant necrosis in hepatocytes cells administered with 400 mg/kg of acetaminophen at 6 hours after acetaminophen administration [16]. Similar study findings with a dose of 3 g/kg body weight of acetaminophen showed that acetaminophen induced necrosis in the hepatocytity groups in rats [40]. This study however never analyzed the number of apoptotic cells for this study. Hence, further studies need to be done to evaluate the influence of increasing dosages of acetaminophen on the formation of apoptotic cells.

**Urtica Dioica**

**General properties**

*Urtica dioica* L. belongs to the genus Urticaea and it is commonly known as the “Stinging Nettle”. The plant is a perennial herb and it is locally referred to as “Thabil” (Kikuyu), “Isambakhuku” (Luyha) and “Isalu” (Wanga) in the Kenyan native languages. *Urtica dioica* grows often along the pathways, fields and wildwood and it is also found between cultivated plants, water tunnels and sometimes streets [47]. It also grows in lighted places in nutrient rich zones and within climatic zones that are hot and mild. *Urtica dioica* plant has been cultivated in many places in the world such as the black sea and Turkey. In historical times, *Urtica dioica* was first recorded in 1562 and identified as herbal medicine by Paracelsus and Matthiolus [48,49]. It is used in the making of drugs, food, dye, fiber and for its cosmetic value [47]. Stinging nettles is most commonly used as a food and the roots, stalk, and leaves are used in the making of tea; the stalk and the leaves are used as vegetables and in salads and also in traditional medicine.

*Urtica dioica* has dark green leaves, roots and stem. The leaves are 2 - 4cm long, oval and are serrated. They also have the stinging nettles that gives a burning effect on the skin when it is touched [47]. The medicinal benefits of the plant has been confirmed through research and positive effects of the phenolic compounds has been established in the management of the coronary heart disease, high blood pressure, cancer, inflammatory diseases, viral and parasitic diseases and as well as the psychotic disorders [50-56]. It has also been shown to be effective in the management the GIT diseases, rheumatism. The *Urtica dioica* "Stinging nettle" has been established to serve various functions in the treatment of diabetes, as an astringent, antirheumatic, diuretic, antiudriatic and cholagogue [51].

Studies have shown that there are various phytochemicals in *Urtica dioica* [56-59]. They act as free radical scavengers, therefore providing hepatoprotection [60]. More precisely, the polyphenols have been indicated to inhibit the cytochrome P-450 enzymes [61]. It has also been shown that the nettle contains fatty substances, albumins, and protein in dry matter [47]. Seeds of nettle contain fixed oil. It has been estimated that a kilogram of fresh nettle plant contains approximately 130mg of vitamin C, 730 mg pro-vitamin A (carotene), Xanthophyll, and siosterin, vitamin B1, K, oxalate. Similarly, formic acid, acetylcione and histamine have been found in the stinging hairs. Ashes generated from nettle are rich in iron oxide, potassium, calcium, and siliconium among others [47]. Minerals are also hepatoprotective in acetaminophen poisoning [62]. Zinc, selenium, and magnesium determined in *Urtica dioica* and suggest great potential of hepatoprotection [62].

*Urtica dioica* and regeneration of the liver

A study was done to investigate the effects of *Urtica dioica* on liver regeneration following partial hepatectomy [46]. The findings showed a decline in the elevated levels of malonyl dialdehyde after partial hepatectomy while the levels of the superoxide dismutase and glutathione were increased following the oral administration of leave extracts of *Urtica dioica*. This study concluded that *Urtica dioica* is beneficial for liver regeneration after liver damage through oxidative stress, proliferation and apoptosis after partial hepatectomy [46].

Similarly, an investigation of the effect of *Nigella sativa* L. and *Urtica dioica* on CCL4 treated rats. Findings showed that rats treated with CCL4 induced damage to the liver and both the peroxidation and the liver enzymes were elevated while the antioxidant enzymes levels were repressed. Treatment with *Urtica dioica* and *Nigella sativa* alone and in a combination lowered the elevated lipid peroxidation and liver enzymes (ALT and AST). Moreover, the antioxidant enzyme levels were elevated. This study concluded that both *Urtica dioica* and *Nigella sativa* alone, and in a combination, protects the liver and increase the antioxidant defense system activity in rats treated with CCL4 [45].

In a different study, it was established that liver damage induced following administration of the acetaminophen at doses of between 100-500 mg/kg could be regenerated following the improvement of the hematological parameters. The study showed that the herbal root extract of Harungana madagascariensis (L) significantly improved (p < 0.05), PCV, Hb and TLC. However, there was a non-significant (p > 0.05) change in the MCV, MCH and MCHC values. In addition, there was significant improvement in the histological tissues. It was concluded that *Harungana madagascariensis* (L) aqueous extracts protects against acetaminophen toxicity [41]. Other studies using different plants *Gnetum africanaum* leaves on biochemical, hematological and histological changes suggested improvement in the liver biochemical, hematological and histological changes in mice. These findings suggested that *Gnetum africanaum* supports the regeneration of the liver [40]. Study findings involving a combination of *Hypericum pertoratum* (HP), *Urtica dioica* (UD) and *Camelia sinensis* (CS) have shown that it can significantly reduce the ameliorated lymphocytes for DNA damage induced by CCH4 [63]. There is currently no data on the same on *Urtica dioica*. 

Data Management for Acetaminophen Toxicological Studies

There are various statistical analytical methods that have been used by studies cited in this work. Oyedee et al. [38] in the assessment of the effect of acetaminophen on the hematological parameters performed a student t-test to compare the results between control and the treated groups. Kanter et al. [45] used one way ANOVA followed by Tukey’s test to compare four different independent treatments (Normal saline + CCl4, CCl4 + Nigella Sativa, CCl4 + Urtica dioica, and CCl4+ Urtica dioica + Nigella Sativa). Piero and Rossoni [64] and Oguz et al. [45] used a similar approach. Pandey et al. [65] and Kandis et al. [66] used a one way ANOVA followed by a Bonferroni adjustment while Ozen and Korkmaz [67] used has also ANOVA and Dunnett’s t-test; a post hoc analysis.

One way ANOVA is used in comparing the means of three or more independent groups that and for testing for the null hypothesis. However, the p-value that is obtained in one way ANOVA is not specific. Hence, post hoc analysis is performed after the one way ANOVA so as to determine the specific difference among the different groups. A range of post-hoc comparison methods are available that differ slightly in their properties. These include Dunnett’s test used for comparing each mean of the different tests with the mean of the control. Other tests includes: Tukey’s Kramer, Fischer, Newman-Keuls tests are used for comparing all means [68]. Tukey’s test also referred to as the honest significant difference (HSD) is the most used post hoc analysis in statistics. It gives the specific significant differences between the independent treatments of the study. Tukey test can only be run when there is significance difference in ANOVA. It is also used as a conservative statistical analysis method when compared to other tests such as Scheffe test in controlling the error rate at p = 0.05 [68]. It is also the useful instead of performing multiple t-tests for individual treatments. In addition, Tukey’s tests is also useful in the comparison of groups that have equal number of values although it can also be applied in the analysis of the treatments that have unequal number of samples/populations in a study [68].

Two-way ANOVA can also be used in the data analysis for toxicological studies; however, it is used in the cases where there is one dependent (measurement variable) and two nominal variables in a study. In this case, it is used in providing solutions to three null hypotheses suggesting that the means of the measurement variable are equal to the different values of the first nominal variable and that the means are also equal for the different values of the second nominal variables as independent variables [69]. In most cases two way ANOVA is followed by Bonferroni adjustment of the values to correct for the errors incorporated in the study.

Multiple comparisons of means in toxicological studies can also be achieved using orthogonal contrasts. This can be used for determining the significance difference for individual means or group means. They can also be applied for determining the linear and nonlinear relationship for toxicological tests at dose and time when equally spaced on some scale [70].

Conclusion

It has been established that acetaminophen induces hepatotoxicity in animal models. There has been no certainty on the precise mechanism of action of acetaminophen toxicity. However, glutathione depletion remains the most referred to mechanism of action of acetaminophen toxicity following the formation of N-acetyl-p-benzoquinoneimine (NAPQI) which binds to glutathione. NAC and other alternative antioxidants have been found to be toxic and expensive to the body. Mineral and phytochemical profiling of Urtica dioica has shown that it has potential in hepatoprotection against acetaminophen. Probably, it may be a better antioxidant compared to NAC and other alternatives. Hence, this warrants further studies on the role of acetaminophen toxicity on the biochemical, hematological and histological changes.

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References


