The efficacy and safety of Acetaminophen for pain relief

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Abstract

Acetaminophen is a non-narcotic analgesic used as an analgesic and antipyretic. Acetaminophen is used for mild to moderate pain; its efficacy is low as analgesic as compared to non-steroidal anti-inflammatory drugs (NSAIDs) as it has no any anti-inflammatory effect. Despite of its well-known use and safely, however; the precise mechanism of acetaminophen still enigmatic. Findings from preclinical studies suggest that the main mechanism of acetaminophen is related to the inhibition of cyclooxygenase 3 (COX-3) which is variant of COX-1 expressed in the brain. However, the profound analgesic antinociceptive effects of acetaminophen cannot depend merely on this pathway. Further findings from preclinical and clinical studies confirmed that acetaminophen and its metabolites can modulate different signaling pain pathways other than COX pathway. Thus, this review revises the potential mechanistic pathways of acetaminophen in relation to its clinical applications.

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Abstract

Acetaminophen is a non-narcotic analgesic used as an analgesic and antipyretic. Acetaminophen is used for mild to moderate pain; its efficacy is low as analgesic as compared to non-steroidal anti-inflammatory drugs (NSAIDs) as it has no any anti-inflammatory effect. Despite of its well-known use and safely, however; the precise mechanism of acetaminophen still enigmatic. Findings from preclinical studies suggest that the main mechanism of acetaminophen is related to the inhibition of cyclooxygenase 3 (COX-3) which is variant of COX-1 expressed in the brain. However, the profound analgesic antinociceptive effects of acetaminophen cannot depend merely on this pathway. Further findings from preclinical and clinical studies confirmed that acetaminophen and its metabolites can modulate different signaling pain pathways other than COX pathway. Thus, this review revises the potential mechanistic pathways of acetaminophen in relation to its clinical applications.

Key words: Acetaminophen, cyclooxygenase 3, COX pathway

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1 INTRODUCTION

Acetaminophen is a 4-aminophenol (Figure 1) also known as paracetamol is a non-narcotic analgesic used as an analgesic and antipyretic. Acetaminophen is used for mild to moderate pain; its efficacy is low as analgesic as compared to non-steroidal anti-inflammatory drugs (NSAIDs) as it has no any anti-inflammatory effect (Ohashi & Kohno, 2020). Clinical evidences for use of acetaminophen in neuropathic pain are insufficient (Freo et al., 2021).

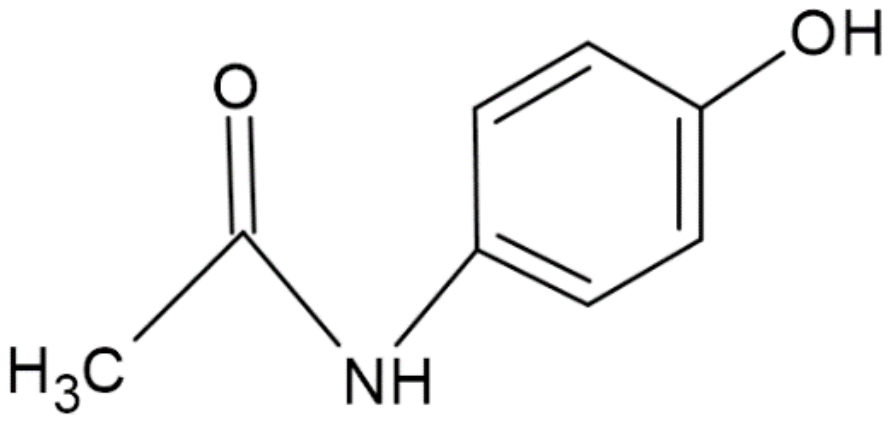


FIGURE 1 Chemical structure of acetaminophen

Acetaminophen was first synthesized in 1877 by Harmon Northrop Mors by reducing of p-nitrophenol by acetic acid (Refat et al., 2017). Despite of strong controversy regarding its safety and efficacy, it was not introduced for clinical use till 1950 when it first introduced as a combination with aspirin and caffeine known as trigesic (Refat et al., 2017). In 1963, acetaminophen became more popular as analgesic drug, and was added to British Pharmacopoeia (Refat et al., 2017; Krenzelok, 2009). Acetaminophen was approved by FDA in 2009 with warring of its combination with other analgesics (Krenzelok, 2009).

Acetaminophen acts either directly by inhibition of cyclooxygenase (COX) enzyme, or indirectly through its metabolite N-arachidonoylphenolamine (AM404) which activate cannabinoid receptors (CB1R and CB2R)

and transient receptor potential cation channel subfamily member 1 (TRPV1) (Ohashi & Kohno, 2020; Ayoub, 2021). Acetaminophen is mainly blocks COX-2 (Kanchanasurakit et al., 2020).

Membrane phospholipid is converted to arachidonic acid (AA) by the action of phospholipase A2 (PLA2), this pathway is inhibited by corticosteroids (Al-Kuraishy et al., 2022). AA by the action of prostaglandin (PG) H2 synthase COX is converted to PGG2, this pathway is inhibited by acetaminophen and NSAIDs (Ferrer et al., 2019). Furthermore, PGG2 is converted to PGH2 by the action of PG H2 synthase peroxidase, this pathway is inhibited by acetaminophen only (Angelis et al., 2021|) (Figure 2).

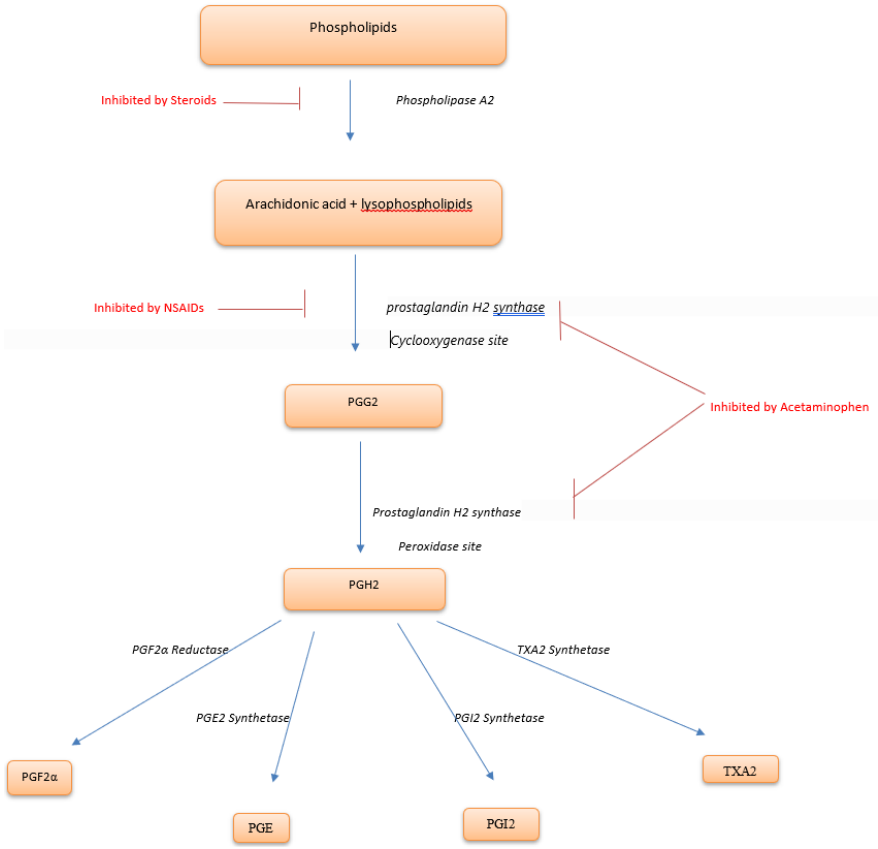


FIGURE 2 Differential effect of acetaminophen and NSAIDs on the COX pathway

Regarding the pharmacokinetic profile of acetaminophen, it orally active and rapidly absorbed from small intestines, its absorption is reduced by food (Souza et al., 2022). Peak plasma concentration of acetaminophen is achieved within 20 minutes during fasting, and within 90 minutes when taken after food (Brookhuis et al., 2021). The bioavailability of acetaminophen is 63-89% depending on used doses, has high volume of distribution about 50L, with minimal plasma protein binding (Spyker et al., 2022).

Prolonged use of acetaminophen is associated with minimal adverse effects, though its use during pregnancy increases risk of asthma in offspring (Shaheen et al., 2019). However, the association between acetaminophen use and risk of asthma is controversial (Sherbash et al., 2020). The recommend daily dose of acetaminophen is 500mg-1g g/day. Higher doses of acetaminophen lead to acute toxicity which causes acute liver failure (Jaeschke et al., 2020). In addition, long-term use of acetaminophen is associated with kidney impairment by 23% and kidney cancer by 28% (Kanchanasurakit et al., 2020). Acetaminophen may interact with different agents and drugs, for example prokinetic drugs accelerate absorption of acetaminophen (Southren et al., 2021). As well, enzyme inducer drugs such as rifampicin enhance acetaminophen toxicity by activating

the formation of N-acetyl-p-benzoquinone imine (NAPQI) (Chowdhury et al., 2020). However, enzyme inhibitors such isoniazid reduces the formation of NAPQI by 70% (Balhara et al., 2021). Of note, 85-95% of acetaminophen is metabolized to non-toxic metabolites, 5-15% is metabolized to toxic metabolites which neutralized by hepatic glutathione (Zacharia & Jacob, 2023). However, 1-2% of acetaminophen is metabolized by deacetylation process to form p-aminophenol which converted by brain fatty acid amide hydrolase (FAAH) to AM404 which has different effect on the brain (Ogemdi, 2019) (Figure 3).

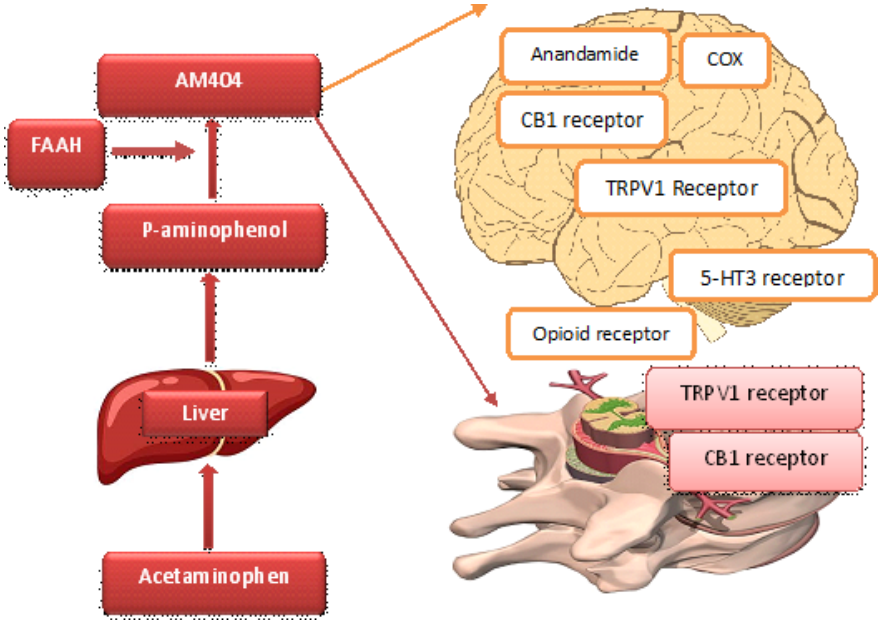


FIGURE 3 Direct and indirect mechanisms

Many studies and reports highlighted that acetaminophen has broad mechanism of action not limited to COX pathway (Ohashi & Kohno, 2020; Kanchanasurakit et al., 2020).Therefore, this review revises the potential mechanistic pathways of acetaminophen in relation to its clinical applications.

ACETAMINOPHEN AND COX-3

COX-3 is a novel COX variant proposed to mediate the action of acetaminophen in both animals and humans. COX-3 encoded gen is differed from that of COX-1 and COX-2 (Ogemdi, 2019). Though, it was suggested that COX-3 is a variant of COX-1 but with different molecular effects (Li et al., 2008). Recently, COX variants are sequenced, and found that COX-3 is a variant of COX-1 (Esh et al., 2021). Acetaminophen can induce analgesia and hypothermia by reducing PGE2 via suppression of COX-3 in mice (Ogemdi, 2019; Li et al., 2008). Acetaminophen can selectively inhibits COX-2 but with very low potency compared to selective COX-2 inhibitors by about 433 fold (Esh et al., 2021). Acetaminophen blocks the peroxidase activity rather than inhibition of COX enzymes (Aminoshariae & Khan, 2015). It has been reported that COX-3 expression is higher in the brain only (Chandrasekharan et al., 2002) that mediate the antipyretic and analgesic effect of acetaminophen. Supporting to this notion, deletion of COX-1 prevents the antipyretic and analgesic effect of acetaminophen in mice (Ayoub & Flower, 2019) suggesting that COX-3 is a variant of COX-1. Likewise, selective COX-3 inhibitors such as antipyrine and aminopyrine produce similar antipyretic and analgesic effects of acetaminophen (Ayoub et al., 2004). An updated experimental study confirmed the expression of COX-3 in knee joint and implicated in the development of arthritis (Biswas et al., 2023). Therefore, COX-3 like COX-1 is peripherally and centrally expressed.

Despite of these preclinical findings, there is strong argument regarding the effect of acetaminophen in relation to COX-3 (Davies et al., 2004; Snipes et al., 2005). The potential conflicting for the functional

activity of COX-3 is related to many points. COX-3 protein is detected in human tissues, though the functional activity of COX-3 enzyme is not sequenced. In addition, many variants of COX-1 are identified in animals and humans, and none of these variants are targeted by acetaminophen (Perrone et al., 2010). As well, COX-1 gene can produce mRNA products that not involved in the degradation process or synthesis of PGs, thus, COX-3 might be inert mRNA products (Mahesh et al., 2010). Furthermore, preclinical studies concerning effect of acetaminophen on COX-3 were inappropriate to be translated in clinical practice due to species difference regarding the expression and activity of COX-1 variants (Kotowska-Rodziewicz et al., 2023). Moreover, acetaminophen inhibits COX-1 when AA concentration is low, and COX-1 knockout mice but not COX-2 knockout mice prevent acetaminophen effect (Graham et al., 2013). Thus, COX-3 is not the potential target of acetaminophen effect.

ACETAMINOPHEN AND SEROTONIN PATHWAY

It has been shown that acetaminophen analgesic effect is mediated by modulating of brain serotonergic neurotransmission (Hamurtekin et al., 2020) and interfering with spinal serotonergic pathway by serotonin antagonist reduces the analgesic effect of acetaminophen in mice (Karandikar et al., 2016). Furthermore, acetaminophen augments serotonin level in the pons and cerebral cortex signifying supra-spinal analgesic effect of acetaminophen (Fukushima et al., 2017). An experimental study demonstrated that intraperitoneal administration of acetaminophen increases serotonin level in the pons by 40% and in the cerebral cortex by 75% through modulation of 5HT2R (Ruggieri et al., 2008). In addition, chronic acetaminophen administration in rats increases serotonin level in the prefrontal cortex, hypothalamus, thalamus and striatum (Blecharz-Klin et al., 2013). Acetaminophen-induced increase of brain serotonin may be through reducing of serotonin metabolism, increasing its release or through inhibition of serotonin reuptake. However, the exact mechanism of acetaminophen effect on brain serotonin is not fully elucidated.

Of interest, acetaminophen metabolite AM404 is generated by FAAH enzyme expressed in brain, dorsal root ganglion and spinal cord (Nilsson et al., 2021). AM404 has 50% analgesic effect of acetaminophen, as AM404 activates 5HT3 but not 5HT1A or 5HT2 which mediate the analgesic effect of acetaminophen (Mallet et al., 2023). Acetaminophen but not AM404 augment brain serotonin (Mallet et al., 2023). In addition, AM404 activates descending serotonergic pathway which has an antinociceptive effect (Kaur, 2020). Presynaptic autoreceptors 5HT1A and 5HT1B inhibit serotonin release from presynaptic neurons. Administration of 5HT1A agonist buspirone blocks the analgesic effect of acetaminophen in mice (Sandrini et al., 2003). Therefore, acetaminophen may act as an antagonist for presynaptic 5HT1A leading to increase serotonin release.

Furthermore, postsynaptic 5HT2 which mediate the excitatory effect of serotonin mediates the analgesic effect of acetaminophen. Systemic administration of 5HT2 antagonist ketanserin attenuates the analgesic effect of acetaminophen but not AM404 (Kose et al., 2019). However, intrathecal administration of ketanserin did not affect the analgesic effect of acetaminophen (Ledebuhr et al., 2022). These findings suggest that 5HT2 mediates the supraspinal but not spinal analgesic effect of acetaminophen. Likewise, spinal 5HT3 mediates the analgesic effect of acetaminophen, and administration of 5HT3 antagonist tropisetron blocks systemic and intrathecal acetaminophen (Irinmwinuwa et al., 2022). However, 5HT3 antagonist ondansetron which act centrally did not affect the analgesic effect of acetaminophen (Libert et al., 2004). Inhibition of spinal 5HT3 by oligodeoxynucleotide did no reduce the effect of acetaminophen (Libert et al., 2004). These findings were confirmed clinically (Pickering et al., 2006; Bandschapp et al., 2011). These findings indicated that 5HT3 mediates supraspinal analgesic effect of acetaminophen.

Moreover, 5HT7 is highly expressed in the brain, involved in antinociception effect, is also mediate the analgesic effect of acetaminophen at spinal level (Kose et al., 2019). A preclinical study found that 5HT7 did not mediate the antipyretic effect of acetaminophen (Hamurtekin et al., 2020). However, preclinical studies demonstrated that the analgesic effect of acetaminophen at spinal level is mediated by activating 5HT7 in the descending antinociceptive serotonergic pathway (Liu et al., 2013; Dogrul et al., 2012).

Therefore, the analgesic effect of acetaminophen at spinal and supraspinal levels is mediated by activation

serotonin release or direct activation of serotonin receptors.

ACETAMINOPHEN AND NITRIC OXIDE PATHWAY

Nitric oxide (NO) is a small molecule widely expressed in the CNS and act as neurotransmitter; it modulates pain transmission negatively or positively (Lundberg & Weitzberg, 2002). Of note, NO and NO synthase are involved in the analgesic effect of low-dose but not high-dose acetaminophen (Angelis et al., 2021). Acetaminophen has ability to inhibit NO synthase in the spinal cord (Godfrey et al., 2007). Therefore, the central analgesic effect acetaminophen could be mediated by suppressing neuronal NO synthase. However, NO-acetaminophen combination was used to reduce acetaminophen-induced hepatotoxicity. NO-acetaminophen has profound analgesic and can reduce neuropathic pain compared to acetaminophen alone (Cooper et al., 2022). Moreover, NMDA receptor produces excitotoxicity by activating the release of NO (Negri et al., 2021). A previous experimental study showed that acetaminophen attenuates substance P and NMDA-mediated spinal hyperalgesia (Björkman et al., 1994). Choi et al (2001) study demonstrated that acetaminophen inhibits spinal nociceptive effect mediated by glutamate and substance P. Therefore; acetaminophen inhibits NO-induced pain transmission. In addition, AM404 inhibits neuronal NO, and release of pro-inflammatory cytokines through inhibition of microglial activation (Costa et al., 2006). Therefore, acetaminophen and its metabolite attenuate NO-induced nociception and pain transmission.

ACETAMINOPHEN AND TRANSIENT RECEPTOR POTENTIAL VANILLOID 1

Transient receptor potential vanilloid 1 (TRPV1) is a non-selective channel receptor triggered by vanilloids and temperature, and mediates the central hyperalgesia (Garami et al., 2020). TRPV1 is highly expressed in nociceptive and sensory neurons, such as sensory C fiber which mediate inflammatory and neuropathic pain (Chang et al., 2021). TRPV1 is mainly present in trigeminal ganglion and dorsal root ganglion (Cha et al., 2020). In the CNS, TRPV1 is expressed in specific brain regions may involve in pain transmission and thermoregulation, such as thalamus, hypothalamus, cerebral cortex, cerebellum, striatum and substantia nigari (Meza et al., 2022). TRPV1 expression is augmented by neuronal injury and inflammation (Meza et al., 2022). Notoriously, TRPV1 agonists inhibit inflammation by reducing the expression of pro-inflammatory TNF-α (Abdel-Salam et al., 2023). Activation of TRPV1 induces the release of different neuropeptides intricate in pain transmission such as somatostatin, substance P (SP) and calcitonin gene related peptide (CGRP) (Messlinger et al., 2020). Therefore, activation of TRPV1 leads to anti-inflammatory and immunomodulatory effects by reducing the release of pro-inflammatory cytokines and induction of neuropeptides. However, TRPV1 antagonists were suggested to be effective for painful conditions, though these agents were withdrawn because of risk of hyperthermia and cognitive impairment (Garami et al., 2020; Caballero, 2022). Thus, TRPV1 agonists were proposed to be effective against pyrexia, diabetic neuropathy, post-herpetic neuralgia and osteoarthritis (Iftinca et al., 2021; Liao et al., 2023). TRPV1 agonist capsaicin is effective in patients with diabetic neuropathy and osteoarthritis (Liao et al., 2023). Therefore, TRPV1 agonists have important anti-inflammatory effects either directly or indirectly by inducing the release neuropeptide such as SP, CGRP and somatostatin (Figure 4).

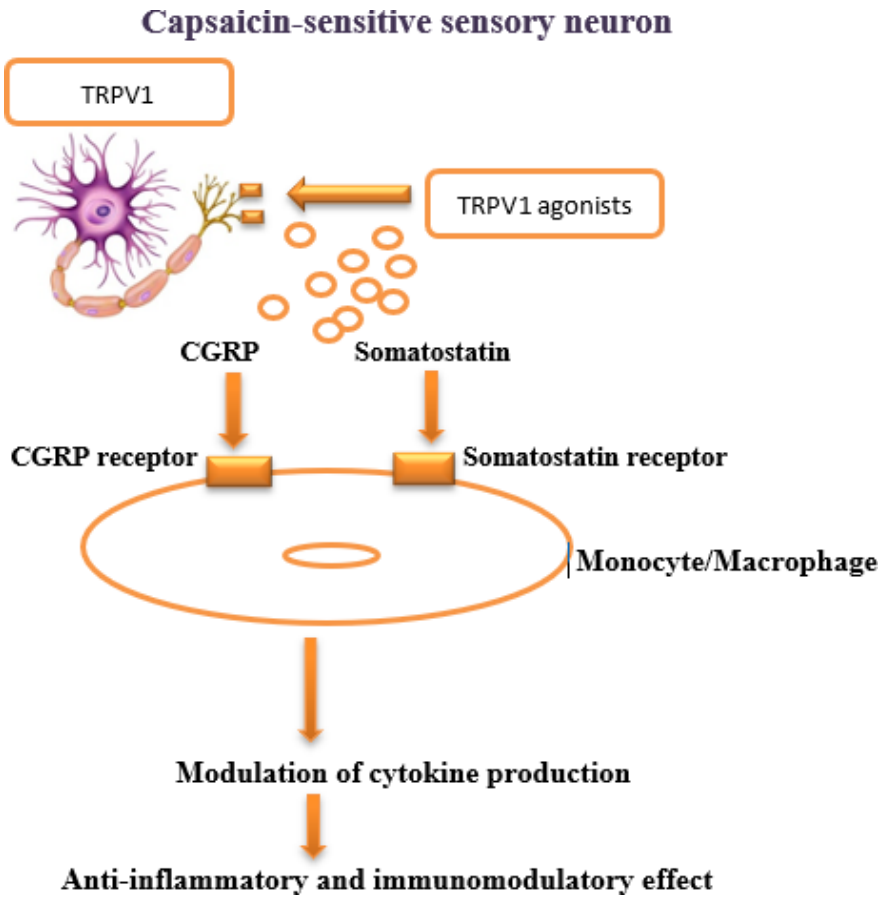
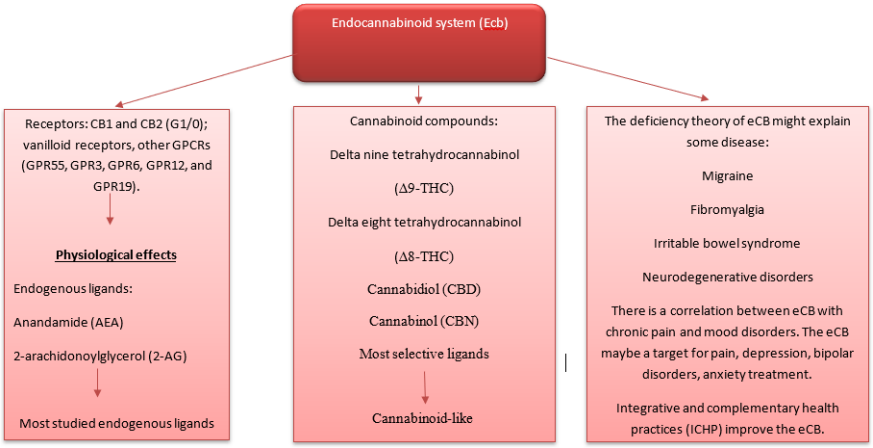


FIGURE 4 TRPV1-mediated anti-inflammatory and immunomodulatory effects

It has been suggested that the central analgesic effect of acetaminophen is mediated by activation of TRPV1 (Mallet et al., 2010). Higher co-expression of TRPV1 and FAAH support this proposition. A preclinical finding confirmed that the antinociceptive effect of acetaminophen is lacking in TRPV1 and FAAH knockout mice. Supporting to this finding, pharmacological suppression of TRPV1 also abolish the antinociceptive effect of acetaminophen (Mallet et al., 2010). As well, the analgesic effect of AM404 is mediated through activation of TRPV1 (Stueber et al., 2018). It has been reported that oral administration of acetaminophen in mice did not affect brain PGE2 level and COX activity, but activates neuronal TRPV1 (Mallet et al., 2010). Furthermore, acetaminophen promotes the activation of supraspinal TRPV1 (Ohashi & Kohno, 2020). Moreover, higher expression of FAAH in the dorsal ganglion and spinal cord, increases biosynthesis of AM404 which activate spinal TRPV1 (Nazıroğlu et al., 2019). In addition, acetaminophen metabolite NAPQI but not acetaminophen activates TRPV1 irreversibly in HEK293 cells (Eberhardt et al., 2017). It has been shown that acetaminophen metabolites including pBQ and NAPQI which are generated by cytochrome P450-dependent acetaminophen metabolism also activate neuronal TRPV1 (Holme et al., 1984). As well, NAPQI has potent agonist effect on ankyrin 1 receptor (Gentry et al., 2015). Remarkably, both acetaminophen and its metabolites stimulate TRPV1 in the inhibitory pain pathway leading to analgesic effects at supraspinal level (Irinmwinuwa et al., 2022). In addition, TRPV4 which is highly expressed in the CNS is involved in the activation of dorsal root ganglion, neuronal hyperexcitability, hyperalgesia and the development of neuropathic pain (Qu et al., 2016). It has been shown that acetaminophen induces analgesic effect by inhibiting TRPV4 which mediate pro-inflammatory and oxidative stress effects, and involved in mechanosensations (Nakagawa et al., 2020). These findings indicated that acetaminophen and its metabolites induce analgesic effects at spinal and supraspinal level via activation of TRPV1 and inhibition of TRPV4.

ACETAMINOPHEN AND ENDOCANNABINOID PATHWAY

Endocannabinoid system is consist of endocannabinoid receptors, ligands and associated enzymes that maintain energy homeostasis, preserve cognitive function and pain control (Al-Kuraishy et al., 2023) (Figure 5).



FiIGURE 5 Components and functional role of endocannabinoid system

Endocannabinoids are endogenous metabolites of various eicosanoid fatty acids, act on cannabinoid receptors (CB1R and CB2R) (Al-Kuraishy et al., 2023) Moreover, endocannabinoids can interacts with other receptors including G-protein coupled receptors (GPRs) and vanilloid receptors (Kilaru & Chapman, 2020). CB1R is mainly expressed in the CNS, whereas CB2R is widely expresses the immune cells. However, CB2R is also expressed in the brainstem neurons and astroglia (Kilaru & Chapman, 2020). Endocannabinoid ligands such as anandamide or arachidonoyl ethanolmide (AEA) and 2-arachidonoyl glycerol (2-AG) are identified. AEA is synthesized from N-arachidonoyl-phosphatidylethanolamine (NAPE) via phospholipase A2, and degraded by fatty-acid amide hydrolase (FAAH) (Martinez Ramirez et al., 2023). 2-AG is generated from diacylglycerol by diacylglycerol lipase, and then converted to AA by monoacylglycerol (MGL) (Kilaru & Chapman, 2020) (**Figure 6**).

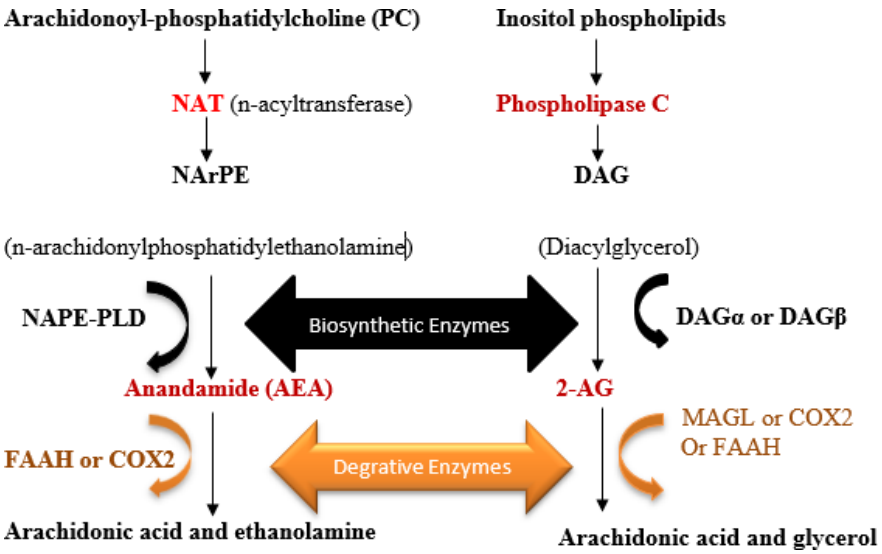


FIGURE 6 Endocannabinoid pathway

Of note, endocannabinoids are rapidly synthesized on demand, and act in paracrine and autocrine fashions (Boczek & Zylinska, 2021). It has been shown that the analgesic effect of acetaminophen is mediated through modulation of endocannabinoid system (Topuz et al., 2020). In addition, AM404 activates supraspinal CB1R with subsequent reinforcement of descending serotonergic inhibitory pathway (Elmer, 2021). Of note, AM404 inhibits reuptake of AEA increasing the central activity of endocannabinoid system (Scienza-Martin et al., 2022). Both acetaminophen and AM404 inhibit FAAH leading to increasing of AEA level (Mallet et al., 2023). However, FAAH is necessary for conversion of acetaminophen to AM404 which has a potent TRPV1 agonist effect (Barrière et al., 2013). Acetaminophen has been reported to exerts anxiolytic effects by activating CB1R (Mageed et al., 2022), however CB1R antagonists does not block the analgesic effects of acetaminophen since this drug acting on multiple pathway in the CNS. A preclinical study confirmed that CB2R antagonist did not affect the analgesic effect of acetaminophen (Mallettet al., 2008) suggesting that acetaminophen effect is mediated by CB1R but not by CB2R. Furthermore, acetaminophen inhibits reuptake of anandamide or AEA leading to mild anxiolytic effect. Inhibition of FAAH which involved in the metabolism of acetaminophen to AM404 in the CNS attenuates the analgesic effect of acetaminophen (Zaitone et al., 2012) signifying that AM404 is mainly involved in the anxiolytic effect of acetaminophen.

It has been shown that acetaminophen has a neuroprotective effect against brain ischemic-reperfusion injury by activating CB1R in rat model (Mageed et al., 2022). Notably, endocannabinoid system is implicated in the pathophysiology of autism spectrum disorder as peripheral and central dysregulated CBRs and enzymes are found in patients with autism spectrum disorder. Therefore, acetaminophen through interaction with endocannabinoid system may induce the development of autism spectrum disorder (Schultz et al., 2021). Thus, prolong use of acetaminophen may increase risk of autism spectrum disorder. Moreover, an in vitro study demonstrated that acetaminophen and its metabolites exert toxic effects on developing mouse cortical neurons by inducing neuronal apoptosis through activating CB1R (Schultz et al., 2012). Therefore, acetaminophen use in children as antipyretic and analgesic with traumatic brain injury may increase risk of cognitive impairment. Of interest, acute acetaminophen intoxication not only induces acute liver failure but also causes acute neurotoxicity by inducing brain oxidative stress and injury of dopaminergic neurons (Vigo et al., 2019).

Therefore, endocannabinoid system is regarded a critical pathway for the analgesic and antinociceptive effects of acetaminophen. However, endocannabinoid system may mediate the neurotoxic and neurodetrimental effect of acetaminophen. Through this pathway acetaminophen has as a double-sward effect could be beneficial or detrimental.

ACETAMINOPHEN AND NEUROTRANSMITTERS

It has been illustrated that prolong use of acetaminophen may affect brain neurotransmitters as confirmed by different studies. A preclinical study showed that use of acetaminophen for 8 weeks leads to behavioral and learning changes in rats by alternating many neurotransmitters in prefrontal cortex (Blecharz-Klin et al., 2013). In addition, augmentation of dopaminergic neurotransmission by L-DOPA and bromocriptine potentiate the analgesic effect of acetaminophen in experimental studies (Bhagyashree et al., 2017). Furthermore, AM404 improves dopaminergic neurotransmission by increasing of anandamide and reducing of nitric oxide (NO) (Oz et al., 2010). Furthermore, endogenous opioid receptors can mediate the spinal and supraspinal analgesic effect of acetaminophen [105]. Backup to notion, administration of opioid receptor antagonist naltrindol abolish the analgesic effect of acetaminophen in an animal model study (Raffa et al., 2004). However, a pilot study showed that opioid receptor antagonist naloxone did not abolish the analgesic effect of acetaminophen in normal healthy volunteers (Pickering et al., 2013) suggesting that opioid system is not involved in the central antinociceptive effect of acetaminophen. Though, opioid system is upregulated during fever by the effect of IL-6, and opioid system also contributes in fever and thermoregulation (Benamar et al., 2002). Thus, low basal activity of opioid system in healthy state may explain the negative association between acetaminophen and opioid system. Furthermore, AM404 has a neuroprotective effect by inhibiting NMDA-induced neurotoxicity, and glutamate release (Saliba et al., 2019). Therefore, acetaminophen analgesic effect could be mediated through inhibition of glutamatergic neurotransmission.

These findings suggest that acetaminophen analgesic effect may be mediated through modulation of different neurotransmitters such as dopaminergic and glutamatergic neurotransmissions.

Taken together, the analgesic effects of acetaminophen and its metabolites are mediated by various pathways including inhibition of COX pathway, activation of descending inhibitory pathway and endocannabinoid pathway (**Figure 7**).

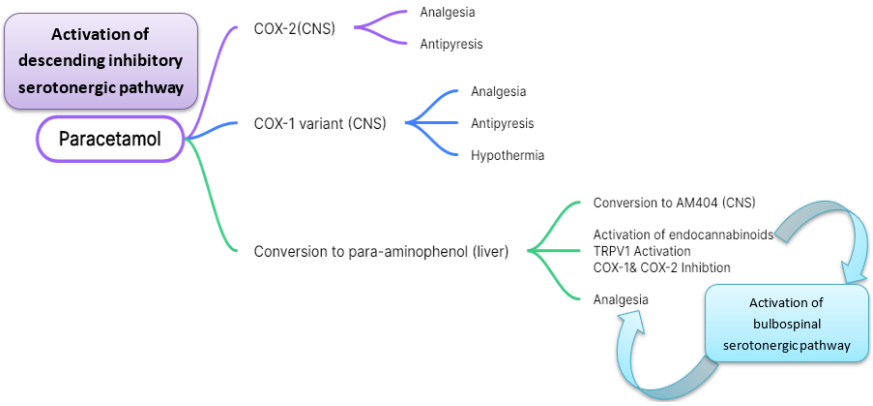


FIGURE 7 Mechanism of acetaminophen

8 CONCLUSION

Acetaminophen is a non-narcotic analgesic used as an analgesic and antipyretic, despite it act on COX it not regarded as a NSAID. Acetaminophen is used for mild to moderate pain; its efficacy is low compared to other NSAIDs as it has no any anti-inflammatory effect. In spite of its famous use and safely, however; the precise mechanism of acetaminophen still mysterious. Findings from preclinical studies suggest that the main mechanism of acetaminophen is related to the inhibition of COX-3 which is a variant of COX-1 expressed in the brain. Though, the substantial analgesic and antinociceptive effects of acetaminophen cannot explain only by COX pathway. Further findings from preclinical and clinical studies confirmed that acetaminophen and its metabolites can modulate different signaling pain pathways other than COX pathway. Acetaminophen acts either directly by inhibition of COX-2 enzyme, or indirectly through its metabolite AM404 which activate CB1R, CB2R, and TRPV1. In addition, acetaminophen improves brain serotonin by reducing of serotonin metabolism, increasing its release or through inhibition of serotonin reuptake. However, the exact mechanism of acetaminophen effect on brain serotonin is not fully elucidated. Therefore, the analgesic effect of acetaminophen at spinal and supraspinal levels may be mediated by activation serotonin release or direct activation of serotonin receptors. Moreover, acetaminophen has ability to inhibit NO synthase in the spinal cord that involved in pain transmission. It has been suggested that the central analgesic effect of acetaminophen is mediated by activation of TRPV1 at spinal and supraspinal level with inhibition of TRPV4. Interestingly, the endocannabinoid system is regarded as a critical pathway for the analgesic and antinociceptive effects of acetaminophen. However, endocannabinoid system may mediate the neurotoxic and neurodetrimental effects of acetaminophen. Through this pathway acetaminophen has as a double-sward effect could be beneficial or detrimental. Finally, acetaminophen analgesic effect may be mediated through modulation of different neurotransmitters such as dopaminergic and glutamatergic neurotransmissions.

Taken together, the analgesic effects of acetaminophen and its metabolites are mediated by various pathways including inhibition of COX pathway, activation of descending inhibitory pathway and endocannabinoid pathway. Despite of these findings the central analgesic effect of acetaminophen still vague, therefore preclinical and clinical studies are warranted in this regard.

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