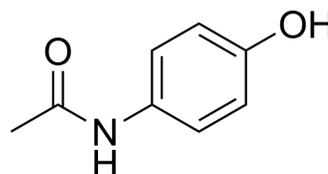


## Acetaminophen

<b>Cat. No.:</b>	HY-66005
<b>CAS No.:</b>	103-90-2
<b>Molecular Formula:</b>	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub>
<b>Molecular Weight:</b>	151.16
<b>Target:</b>	COX; Endogenous Metabolite; Histone Acetyltransferase; Bacterial; Parasite
<b>Pathway:</b>	Immunology/Inflammation; Metabolic Enzyme/Protease; Epigenetics; Anti-infection
<b>Storage:</b>	Powder    -20°C    3 years 4°C        2 years



\* The compound is unstable in solutions, freshly prepared is recommended.

### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : 250 mg/mL (1653.88 mM; Need ultrasonic)  
H<sub>2</sub>O : 10 mg/mL (66.16 mM; Need ultrasonic)

	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM	6.6155 mL	33.0775 mL	66.1551 mL
	5 mM	1.3231 mL	6.6155 mL	13.2310 mL
	10 mM	0.6616 mL	3.3078 mL	6.6155 mL

Please refer to the solubility information to select the appropriate solvent.

#### In Vivo

- Add each solvent one by one: 50% PEG300 >> 50% saline  
Solubility: 66.67 mg/mL (441.06 mM); Clear solution; Need ultrasonic and heat to 30°C
- Add each solvent one by one: 0.5% CMC-Na/saline water  
Solubility: 10 mg/mL (66.16 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: saline >> 0.5% Tween-80  
Solubility: 10 mg/mL (66.16 mM); Clear solution; Need ultrasonic
- Add each solvent one by one: PBS  
Solubility: 6.67 mg/mL (44.13 mM); Clear solution; Need ultrasonic

### BIOLOGICAL ACTIVITY

#### Description

Acetaminophen (Paracetamol) is a selective cyclooxygenase-2 (COX-2) inhibitor with an IC<sub>50</sub> of 25.8 μM; is a widely used antipyretic and analgesic agent<sup>[1][2][3]</sup>. Acetaminophen is a potent hepatic N-acetyltransferase 2 (NAT2) inhibitor<sup>[4]</sup>.

#### IC<sub>50</sub> & Target

COX-2	COX-1
25.8 μM (IC <sub>50</sub> )	113.7 μM (IC <sub>50</sub> )

<b>In Vitro</b>	<p>In vitro, acetaminophen elicits a 4.4-fold selectivity toward COX-2 inhibition (IC<sub>50</sub> 113.7 μM for COX-1; IC<sub>50</sub> 25.8 μM for COX-2). Following oral administration of the drug, maximal ex vivo inhibitions are 56% (COX-1) and 83% (COX-2). Acetaminophen plasma concentrations remain above the in vitro IC<sub>50</sub> for COX-2 for at least 5 h postadministration. Ex vivo IC<sub>50</sub> values (COX-1: 105.2 μM; COX-2: 26.3 μM) of acetaminophen compared favorably with its in vitro IC<sub>50</sub> values. In contrast to previous concepts, acetaminophen inhibited COX-2 by more than 80%, i.e., to a degree comparable to nonsteroidal antiinflammatory drugs (NSAIDs) and selective COX-2 inhibitors. However, a &gt;95% COX-1 blockade relevant for suppression of platelet function is not achieved<sup>[1]</sup>. MTT assay shows that Acetaminophen (APAP) in a dose of 50 mM significantly (p&lt;0.001) reduces cell viability to 61.5±6.65%. Interestingly, the significant (p&lt;0.01) increase in cell viability to 79.7±2.47% is observed in the Acetaminophen/HV110 co-treated cells, compared to Acetaminophen treated cells<sup>[2]</sup>. MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
<b>In Vivo</b>	<p>Administering Acetaminophen (250 mg/kg, orally) to the mice causes significant (p&lt;0.001) liver damage and necrosis of cells as evidenced by the elevated serum hepatic enzymes alanine aminotransferase (ALT), aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (γGT) compared with normal group. Conversely, effects of pretreatment with different doses of citral (125, 250, and 500 mg/kg) exhibited a significant (p&lt;0.05) decrease in serum activities of ALT (91.79%, 93.07%, and 95.61%, resp.), AST (93.40%, 91.89%, and 96.52%, resp.), ALP (39.29%, 37.07%, and 59.80%, resp.), and γGT (92.83%, 91.59%, and 93.0%, resp.), when compared to the Acetaminophen group. Similar results were found in pretreatment with SLM on the activity of ALT (95.90%), AST (95.03%), ALP (70.52%), and γGT (92.69%)<sup>[3]</sup>. MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

## PROTOCOL

<b>Cell Assay</b> <sup>[2]</sup>	<p>Human hepatoma cell line HepG2 is cultured in low glucose DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/mL Penicillin and 100 μg/mL Streptomycin and 2 mM L-glutamine. The cells are maintained in 75 cm<sup>2</sup> flasks at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> and split at 80% confluence every 5 days. Cells are seeded in 24-well plate (2×10<sup>5</sup> cells) and incubated at 37°C overnight followed by cells pretreatment with complete DMEM containing high glucose concentration in order to downregulate autophagy. After 6 h, cells are treated with different concentrations of postbiotics obtained from Lactobacillus fermentum BGHV110 strain (HV110) in order to select appropriate dose for further experiments. Postbiotic is dissolved in complete DMEM medium and added to the cells in specific final concentration. In all other experiments seeded cells are treated with 50 mM Acetaminophen alone or co-treated with 50 mM Acetaminophen and selected dose of lyophilized HV110. To analyze autophagic flux, simultaneously with treatments, cells are exposed to lysosomotropic agent Chloroquine at a concentration of 25 μM, to inhibit autophagosome-lysosome fusion<sup>[2]</sup>. MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
<b>Animal Administration</b> <sup>[3]</sup>	<p>Mice<sup>[3]</sup>  Male Swiss mice (30-40 g) are used. The experimental animals are divided into six groups of five animals each. Firstly, each group receive orally during seven days the following treatment: Group I: the mice do not receive any treatment (normal). Group II: the mice receive citral vehicle (0.1% Tween 80 solution). Groups III-V: the mice are pretreated with citral at doses of 125, 250, and 500 mg/kg, respectively. Group VI: the mice are pretreated with the hepatoprotective standard drug Silymarin (SLM) (200 mg/kg). After this time, the animals fasted for 8 h and then receive oral Acetaminophen on the seventh day at a dose of 250 mg/kg in Groups II-VI. Group I orally receive saline that contained 0.1% Tween 80 solution (Acetaminophen vehicle). The stock solution is used as the first concentration of 50 mg/mL and after that is diluted in 0.1% Tween 80 solution to prepare the solutions of 25 and 12.5 mg/mL. After 12 h of Acetaminophen administration, serum samples and liver tissue are collected followed by biochemistry and histological analysis. MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

## CUSTOMER VALIDATION

- Cell. 2023 Dec 7;186(25):5500-5516.e21.

- Nat Commun. 2021 Sep 20;12(1):5548.
- Nat Commun. 2021 Aug 26;12(1):5131.
- Nat Commun. 2021 May 17;12(1):2863.
- Theranostics. 2017 Sep 26;7(17):4135-4148.

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## REFERENCES

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- [1]. Hinz, B, et al. Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. FASEB J, 2008. 22(2): p. 383-90.
- [2]. Miroslav Dinić, et al. Lactobacillus fermentum Postbiotic-induced Autophagy as Potential Approach for Treatment of Acetaminophen Hepatotoxicity. Front Microbiol. 2017 Apr 6;8:594.
- [3]. Uchida NS, et al. Hepatoprotective Effect of Citral on Acetaminophen-Induced Liver Toxicity in Mice. Evid Based Complement Alternat Med. 2017;2017:1796209.
- [4]. Rothen JP, et al. Acetaminophen is an inhibitor of hepatic N-acetyltransferase 2 in vitro and in vivo. Pharmacogenetics. 1998 Dec;8(6):553-9.
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**Caution: Product has not been fully validated for medical applications. For research use only.**

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