



Inclusion of biopiperine in the kappa-carrageenan complex might improve its bioaccessibility and *in vivo* anti-inflammatory activity in edema-induced Wistar rats

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ARTICLE INFO

Received on: 08/01/2020
Accepted on: 12/04/2020
Available online: 05/06/2020

Key words:

Anti-inflammatory,
Carrageenan, *Piperaceae*,
piperine.

ABSTRACT

Piper nigrum has been used in Indonesian traditional medicine to alleviate pain. Piperine, a nitrogenous substance isolated from the plant, has been reported for its anti-inflammatory activity. However, this compound is slightly soluble in water, which impacts its bioaccessibility. A recent study reported that a co-ground mixture of piperine and β -cyclodextrin revealed a significant increase of dissolved piperine at 15 minutes of dissolution test compared to that of pure piperine. This work was aimed to study the bioaccessibility of the carrageenan-complexed piperine in Wistar rats and assayed its anti-inflammatory activity on the edema-induced paw of the rats. Both isolated (from *P. nigrum*) and synthetic (TCI, Tokyo Chemical Industry) piperines were used as the standards for the bioaccessibility assay, whereas acetosal was the standard drug for the anti-inflammatory activity study. The carrageenan-complexed piperine revealed a better bioaccessibility ($C_{max} = 0.34 \mu\text{g/ml}$; T_{max} at 30 minutes) than that of the isolated piperine ($C_{max} = 0.12 \mu\text{g/ml}$, T_{max} at 60 minutes), whereas the synthetic piperine showed the best absorption ($C_{max} = 0.48 \mu\text{g/ml}$, T_{max} at 30 minutes). The anti-inflammatory activity of carrageenan-complexed piperine at a dose of 393 mg/kg body weight (BW) (contains 100 mg of piperine) equals to the acetosal dose of 45 mg/kg BW. Thus, the inclusion of biopiperine in the carrageenan complex might improve its bioaccessibility and *in vivo* anti-inflammatory activity in Wistar rats.

INTRODUCTION

Piper nigrum (black pepper; *Piperaceae* family) and its active component piperine as shown in [Figure 1](#) have been used in Indonesian traditional medicine to alleviate pain in the neck and throat. The medicine is usually prepared by mixing a teaspoon of honey with a few amounts of black pepper in a half cup of warm water.

A recent study reported that piperine, a nitrogenous substance contained in the fruit of this plant, at doses of 10 and 15 mg/kg body weight (BW) exerted anti-inflammatory effect after 30 minutes (and lasted in 60 minutes) on carrageenan-

induced paw inflammation in rats, whereas the hexane and ethanol extracts of *P. nigrum* produced a similar activity at 10 mg/kg BW but lasted for longer time ([Tasleem et al., 2014](#)). The dichloromethane fraction of *P. nigrum* (dose of 100 mg and 200 mg/kg BW) exhibits anti-inflammatory activity by reducing the production of IL-1 β , IL-6, and TNF- α in the cerebral cortical and hippocampal tissues of rats ([Wang et al., 2017](#)). Piperine inhibits the activation and translocation of NF-kappaB. This pungent compound blocks the phosphorylation and degradation of IkappaBa by attenuating TNF- α induced IkappaB kinase activity in endothelial cells ([Kumar et al., 2007](#)). Moreover, this compound significantly showed an anti-inflammatory effect by inhibiting the expression of IL6 and MMP13 and reduced the production of PGE2 in a dose-dependent manner (10–100 $\mu\text{g/ml}$) ([Bang et al., 2009](#)).

Piperine is a very weak base, which can be hydrolyzed by acid or base to volatile basic piperine (piperidine; C₅H₁₁N) and piperic acid (C₁₂H₁₀O₄). However, this compound is slightly

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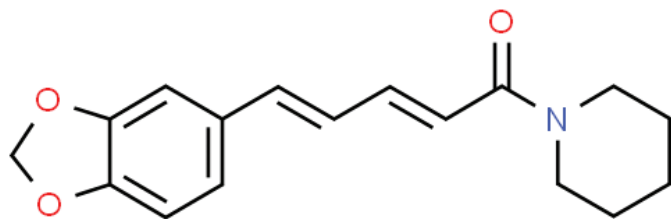


Figure 1. 2D structure of piperine (C₁₇H₁₉NO₃; the IUPAC name 1-(5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadienyl) piperidine; ChemSpider ID 553590).

soluble or practically insoluble in water, which impacts its bioaccessibility. This insolubility of piperine underlies the development of piperine-contained preparations, which includes nanoformulations and encapsulations in lipid bodies (Gorgani *et al.*, 2017). Similarly, another method, e.g., complex formation, is widely used to increase both the water solubility and the stability of hydrophobic drugs. The complexing compounds that are often used in this technique are α -, β -, γ -cyclodextrin (contain 6, 7, or 8 dextrose molecules bound in a 1,4 configuration to form rings of various diameters) (Patel *et al.*, 2017). A recent study reported that a co-ground mixture of piperine and β -cyclodextrin (molar ratio of 1:1) revealed a significant increase (16 times) of dissolved piperine at 15 minutes of dissolution test compared to that of pure piperine (Ezawa *et al.*, 2016). Nonetheless, there is a lack of such a study on the inclusion of piperine in carrageenan, and thus, this work studied the bioaccessibility of the carrageenan-complexed piperine in Wistar rats and assayed its anti-inflammatory activity on the edema-induced paw of the rats.

MATERIALS AND METHODS

Materials

The piperine (isolated from *P. nigrum*) and carrageenan-complexed piperine (prepared by mixing isolated piperine with kappa-carrageenan paste) were provided by the colleagues at the Indonesian School of Pharmacy. The pure standard piperine was purchased from Tokyo Chemical Industry (TCI CAS RN 94-62-2; Product No. P0460). All other chemicals, e.g., sterile bidistilled water (Ikapharmindo, Indonesia), methanol high performance liquid chromatography (HPLC) grade (Fulltime), κ -carrageenan (Sigma Aldrich), and other chemicals were of analytical grade and used without further purification.

Animals

A total of 65 healthy Wistar white male rats, age 2–3 months, weight 200–250 g, were purchased from D45 White Rats Experimental Animal Supplier, West Java, Indonesia, and were identified its strain at the Laboratory of Taxonomy, Department of Biology, Faculty Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia (Document No. 199/10/HB/2018). The rats were acclimatized for 7 days before treatment and housed in cages (30 cm × 24 cm × 10 cm) consisting of three animals per cage under standard laboratory conditions (26°C ± 2°C; 12

hours light/dark cycles). The rats were given free access to food and drink. All the experimental protocols conducted on rats were performed by following the internationally accepted principles for laboratory animal use and care and were approved by the Universitas Padjadjaran Research Ethics Committee (No. 1426/UN6.KEP/EC/2018).

Instruments

UV-visible spectrophotometer (Shimadzu UV-1800), RP-HPLC (Waters 1525 Binary HPLC) with C18 column (Phenomenex), digital plethysmometer (PLM-01 Plus), and digital analytical balance (Mettler Toledo Dragon 204) were used.

Determination of λ_{max} of piperine and validation of the HPLC analytical method

Accurately weighed 10 mg of standard piperine/isolated piperine/carrageenan-complexed piperine was dissolved in methanol analytical grade to obtain 1,000 μ g/ml. All piperine solutions were measured in methanol with a UV spectrophotometer scanning from 200 to 380 nm to obtain the λ_{max} (Fig. 2). The solutions were diluted to serial concentrations and were injected into the C18 HPLC column (mobile phase: methanol–water 70:30; flow rate 10 minutes/minute) to calculate the standard curves. The validation of the analytical method was performed by employing the method proposed by Upadhyay *et al.* (2013).

Determination of C_{max} and T_{max} of carrageenan-complexed piperine in Wistar rats

The procedure employed was based on that of Shao *et al.* (2015) with a few modifications. All rats have fasted for 18 hours (with only free access to water) before drug administration. The rats were randomly divided into: (1) group I (n = 15; three rats for each time of sampling) was treated with piperine isolated from *P. nigrum* dose of 100 mg/kg BW, (2) group II (n = 15; three rats for each time of sampling) was treated with carrageenan-complexed piperine dose of 393 mg/kg BW (equivalent to piperine 100 mg/kg BW), and (3) group III (n = 15; three rats for each time of sampling) was treated with standard piperine dose of 100 mg/kg BW. All test drugs, e.g., piperine isolate, piperine standard, and carrageenan-complexed piperine, were administered to the rats by using oral gavage feeder. The blood samples (0.3 ml) were collected into the heparinized tubes from rats in each group at 0, 15, 30, 60, and 90 minutes. The blood was centrifuged for 15 minutes at 5,000 rpm (2,884 rcf; rotor diameter = 86 mm), and the supernatant was added with methanol and vortex-homogenized for 1 minute to precipitate the proteins. Finally, an aliquot of 20 μ l of the methanolic plasma was injected into the C18 HPLC column (mobile phase: methanol–water 70:30; detection at 340 nm; and flow rate of 10 minutes/minute) (Shao *et al.*, 2015). The maximum plasma concentration (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were compiled from the blood data. The area under the drug concentration versus time curve was calculated from 0 to 90 minutes using the linear trapezoidal method.

Anti-inflammatory activity of carrageenan-complexed piperine in inflammatory-induced Wistar rats

The anti-inflammatory activity assay was carried out by applying a method proposed by Bang *et al.* (2009). All rats have fasted for 18 hours (with only free access to water) before drug administration. The rats were randomly divided into: (1) group I ($n = 4$), the negative control was treated with Arabic gum suspension 2%; (2) group II ($n = 4$), the positive control was treated with acetosal dose of 36 mg/kg BW; (3) group III ($n =$

4) was treated with piperine isolated from *P. nigrum* dose of 100 mg/kg BW; (4) group IV ($n = 4$) was treated with carrageenan-complexed piperine dose of 393 mg/kg BW (equivalent to piperine 100 mg/kg BW); and (5) group V ($n = 4$) was treated with piperine dose of 100 mg/kg BW. After 1 hour, carrageenan 1% was injected subcutaneously on the sole of the rat's left foot. The measurement of edema was carried out at 0th-, 1st-, 2nd-, 3rd-, 4th-, 5th-, and 6th-hour post-inflammatory inducing by using a digital plethysmometer (PLM-01 Plus).

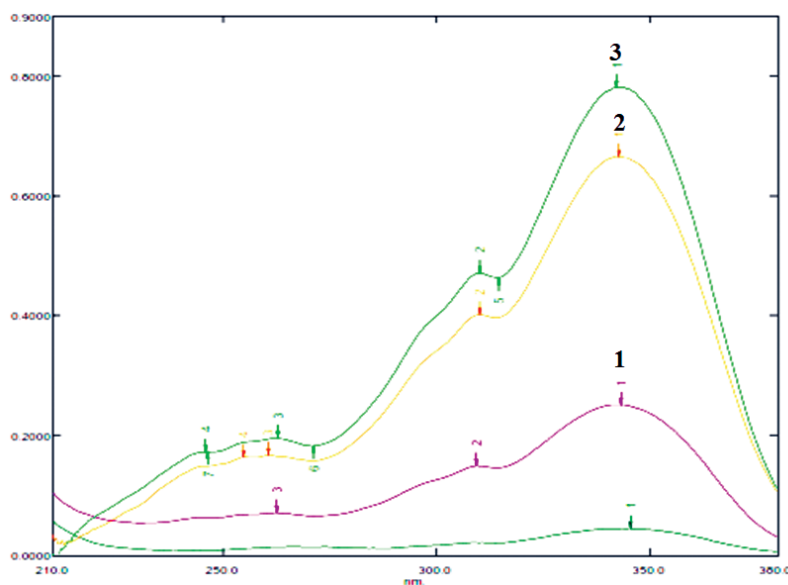


Figure 2. The absorption spectra of carrageenan-complexed piperine (1), piperine isolated from *P. nigrum* (2), and standard piperine (3), in methanol analytical grade. The three samples show maxima at 342 nm.

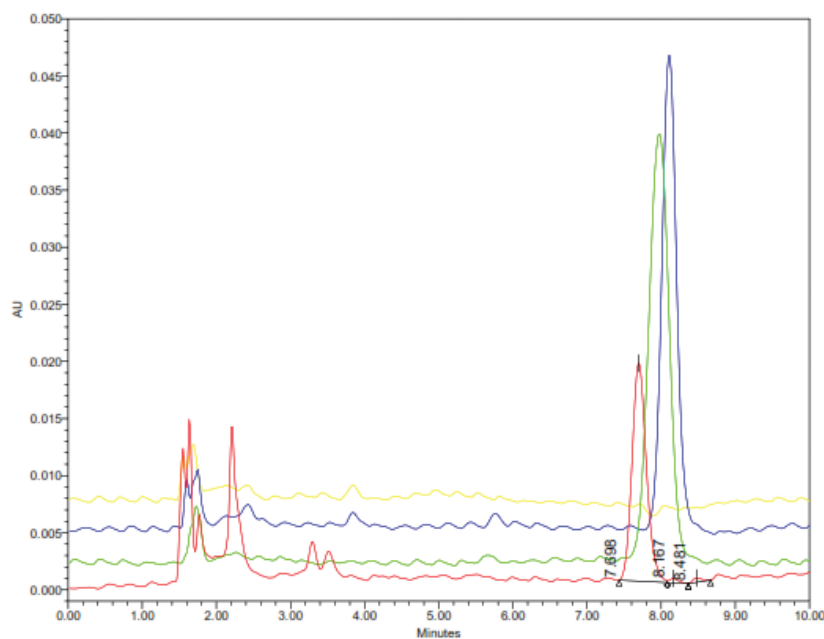


Figure 3. The HPLC chromatograms of rat's blood plasma (blank; yellow), carrageenan-complexed piperine (red; $t_R = 7.698$ minutes), piperine isolated from *P. nigrum* (green; $t_R = 8.167$ minutes), and standard piperine (blue; $t_R = 8.481$ minutes), in methanol HPLC grade.

RESULTS AND DISCUSSION

The carrageenan-complexed piperine shows the maximum absorbance at 342 nm (Fig. 2) similar to that of piperine isolated from *P. nigrum* and standard piperine. This result corresponds with a previous study in Indian polyherbal formulations (Singh *et al.*, 2011).

The carrageenan-complexed piperine is eluted at a shorter time (Fig. 3) than the isolate and the standard, due to its more polar property. This polar property is contributed by the kappa-carrageenan that possesses one sulfate substituent at C4 on the β -linked D-galactose residues. Kappa-carrageenan contains 25%–30% of sulfate ester and 28%–25% of anhydrogalactose units (Barbeyron *et al.*, 2000). A previous study reported that an increase of piperine solubility was observed after this substance was co-ground with β -cyclodextrin in a molar ratio of 1:1. This more polar property was attributed to the interaction between the aromatic ring of piperine (which is lipophilic) and the cavity of β -cyclodextrin (Ezawa *et al.*, 2016).

Validation of the HPLC analytical method revealed that the calibration curve for carrageenan-complexed piperine was linear over the range of 0.05–0.1 $\mu\text{g/ml}$ in plasma ($y = 728,755x + 3,683.4$; $r = 0.9947$) with the lower limit of detection of 0.01315 and the lower limit of quantification of 0.04384 $\mu\text{g/ml}$. Meanwhile, the method proved a good accuracy (the extraction recovery was 100.00%) for carrageenan-complexed piperine.

Carrageenan-complexed piperine, given as a single oral dose of 393 mg/kg BW, could be quantified in the rat's plasma. The maximum concentration (C_{max}) was 0.34 $\mu\text{g/ml}$ at 30 minutes, which is better than that of the isolated piperine from *P. nigrum* ($C_{\text{max}} = 0.12 \mu\text{g/ml}$ at 60 minutes). The standard piperine indicated the best bioaccessibility ($C_{\text{max}} = 0.48 \mu\text{g/ml}$ 30 minutes). However, the inclusion of piperine in the carrageenan complex resulted in faster elimination as proven by its plasma concentration at 60th minute compared to the other piperine (Fig. 4).

Despite its low solubility in water, the pharmacokinetics of a single oral dose of piperine (given as 100 mg of Benjakul tablet of Thai medicine) in 20 healthy Thai subjects revealed that $C_{\text{max}} = 467 \text{ ng/ml}$ was reached at 1 hour (Jumpa-ngern *et al.*, 2013). An intravenous administration of piperine lipid nanoparticles, positively charged stearyl amine, and pegylated lipid nanospheres (LN-P-PEG) of piperine in BALB/c mice showed that LN-P-PEG of piperine possessed the best pharmacokinetic parameters (Veerareddy and Vobalaboina, 2008).

The carrageenan-complexed piperine significantly reduces the edema on the inflammatory-induced paw rats (Fig. 5) compared to that of the negative control ($p = 0.017$). The anti-inflammatory activity of carrageenan-complexed piperine at a dose of 393 mg/kg BW (equivalent to 100 mg piperine) is not significantly different from that of the acetosal dose of 45 mg/kg BW in rats ($p = 0.315$).

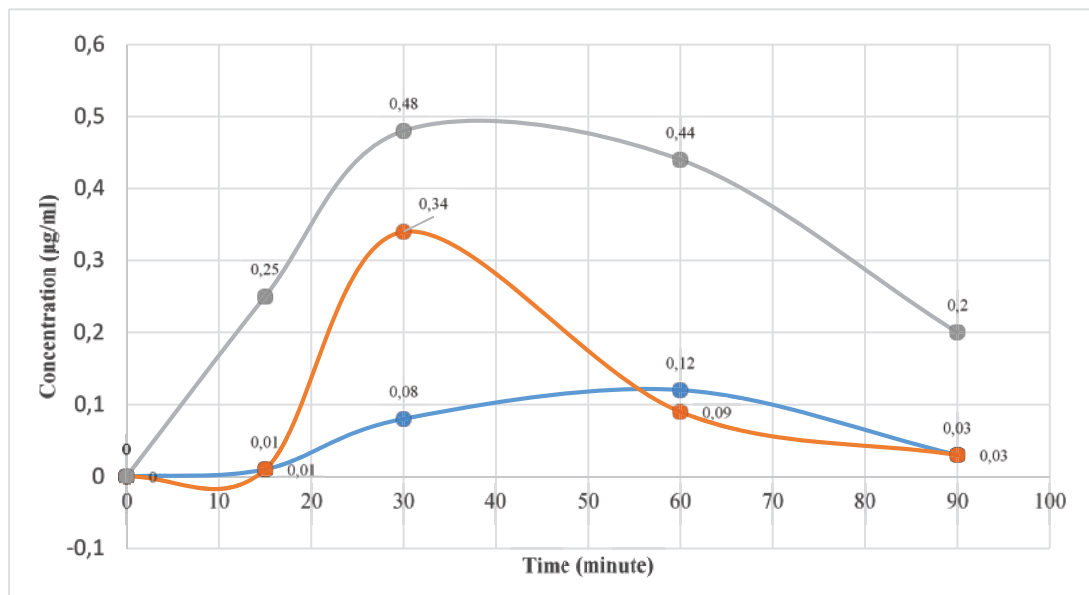


Figure 4. The curve of piperine concentration in rat's blood plasma plotted against time.

Carrageenan-complexed piperine (red; $C_{\text{max}} = 0.34 \mu\text{g/ml}$ at 30 minutes), isolated piperine from *P. nigrum* (blue; $C_{\text{max}} = 0.12 \mu\text{g/ml}$ at 60 minutes), and standard piperine (gray; $C_{\text{max}} = 0.48 \mu\text{g/ml}$ at 30 minutes).

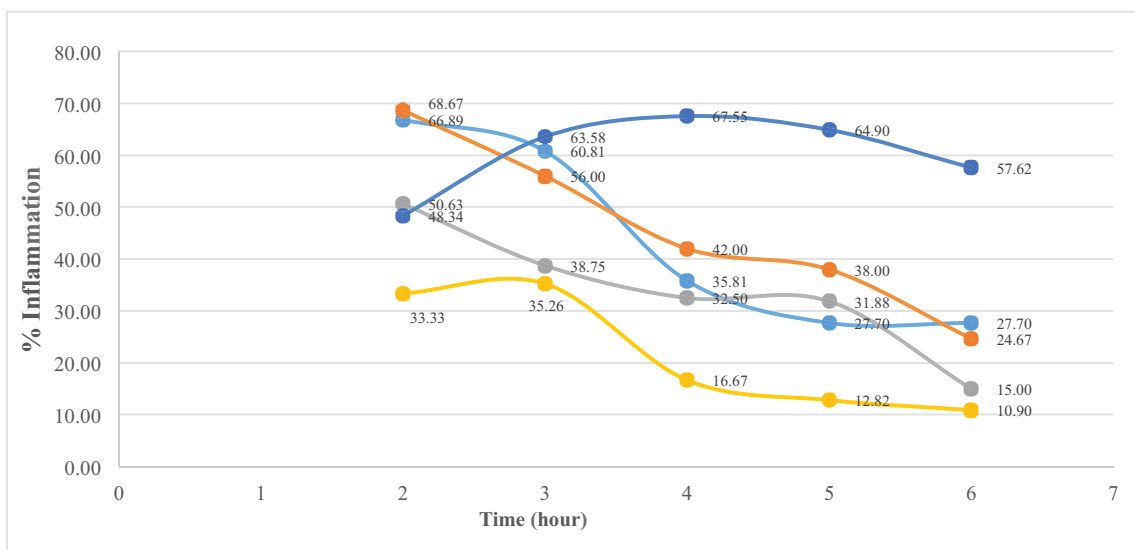


Figure 5. The anti-inflammatory activity calculated as the percentage of inflammation against time.

Carrageenan-complexed piperine is represented as a gray curve, the isolated piperine is the orange curve, and standard piperine is the light blue curve; acetosal: the positive control is the yellow curve; negative control is the dark blue curve.

CONCLUSION

The inclusion of piperine in the carrageenan complex given as a single oral dose of 393 mg/kg BW could be quantified in the rat's plasma. The maximum concentration (C_{max}) was 0.34 $\mu\text{g/ml}$ at 30 minutes, which is better than that of the isolated piperine from *P. nigrum* ($C_{max} = 0.12 \mu\text{g/ml}$ at 60 minutes). This carrageenan-complexed piperine gave a faster elimination as proven by its lowest plasma concentration at 60th minute compared to the other piperines. The anti-inflammatory activity of carrageenan-complexed piperine at a dose of 393 mg/kg BW (equivalent to 100 mg piperine) is not significantly different from that of the acetosal dose of 45 mg/kg BW in rats.

ACKNOWLEDGMENT

The authors would like to thank the Indonesian School of Pharmacy (Sekolah Tinggi Farmasi Indonesia) for funding the publication fee of this article.

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How to cite this article:

Simanjuntak N, Levita J, Subarnas A. Inclusion of biopiperine in the kappa-carrageenan complex might improve its bioaccessibility and *in vivo* anti-inflammatory activity in edema-induced Wistar rats. *J Appl Pharm Sci*, 2020; 10(06):039–043.