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Research Article

Antiplatelet, Analgesic, and Anti-inflammatory Effects of Piperine are Mediated Through Several Different Mechanisms

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Abstract

Piperine is a phytocompound found in black and long pepper (*Piper nigrum* and *Piper longum*, respectively). It has several important pharmacological properties. The current study aimed to evaluate piperine for analgesic, anti-inflammatory, and anti-platelet activities. The analgesic and anti-inflammatory effects were assessed in mice and rats, respectively, using acetic acid and formalin-induced nociception, and carrageenan-induced rat paw edema tests. The intraperitoneal (i.p.) administration of Piperine (10, 50, and 100 µg/kg) produced significant inhibition ($P < 0.01$) of the acetic acid-induced writhing in mice and suppressed formalin-induced licking response of animals. Piperine (10, 50, and 100 µg/kg) produced a marked anti-inflammatory effect in carrageenan-induced rat paw edema assay comparable to diclofenac and produced a dose-dependent (1,5, and 10µM) inhibitory effect against arachidonic acid and adenosine diphosphate-induced platelet aggregation. However, piperine was less potent against the platelet aggregation induced by the platelet-activating factor and epinephrine. These data suggest that piperine possesses peripheral analgesic and anti-inflammatory properties, with potent antiplatelet effects against arachidonic acid and adenosine diphosphate.

Keywords: Piperine, platelet aggregation, analgesic, anti-inflammatory, arachidonic acid, epinephrine

1. Introduction

Piperine, an important alkaloid found in *Piper nigrum* (Piperaceae) and *Piper longum* (Piperaceae), has demonstrated a range of biological activities, such as anti-degenerative, anti-angiogenesis, anti-cancer, and antioxidant properties. Additionally, it has been shown to improve the bioavailability of certain drugs (Meghwal and Goswami 2013, Doucette et al. 2013). Further research indicates that piperine also has anti-inflammatory effects, which it exerts by inhibiting the generation of prostaglandin E2 (PGE2) through the suppression of COX-2 gene expression and protein synthesis (Vaibhav et al. 2012, Umar et al. 2013). In relation to its potential anti-platelet effects, both piperine and piperine-enriched ethanol extracts from *Piper longum* exhibit the ability to suppress platelet aggregation *in vitro* (Raghavendra and Naidu 2009), though the exact mechanism behind this action remains unclear.

Inflammation has a seminal role in the pathogenesis of several diseases such as diabetes, heart disease, arthritis, chronic obstructive pulmonary disease, and many more. Therefore, it is important to keep searching for safer and more efficacious anti-inflammatory agents, especially from natural sources. Similarly, platelet aggregation is a rapid and complex process that leads to the formation of hemostatic plugs and arterial thrombi. These

thrombi are key contributors to thromboembolic conditions like stroke, heart attack, atherosclerosis, and peripheral vascular disease. Various agonists (such as adenosine diphosphate (ADP), arachidonic acid (AA), epinephrine, platelet-activating factor (PAF), and collagen), especially AA can activate platelets. This activation triggers a series of events that result in the enzyme-mediated AA breakdown (Hirsh 1987, Armstrong 1996, Siess 1989). The release of AA from the cell membrane is mediated by phospholipase A_2 (PLA_2) and is subsequently metabolized by cyclooxygenases (COX) and thromboxane A_2 (TXA_2) synthase, producing eicosanoid products such as thromboxane (TX), prostaglandins (PGs), and other oxygenated derivatives (Siess 1989, Kuehl Jr and Egan 1980). Activated macrophages play a role in promoting the inflammatory response by generating pro-inflammatory compounds derived from eicosanoids. This process is driven by the stimulation of the arachidonic acid (AA) metabolic cascade, which also contributes to the regulation of platelet aggregation. As a result, focusing on the enzymes responsible for AA metabolism has become a promising therapeutic approach for managing and treating conditions such as thrombosis and chronic inflammatory diseases (Frolov et al. 2013, Panara et al. 1999, Moscardó et al. 2013, Knijff-Dutmer et al. 2002). This study aimed to explore the effects of piperine on platelet aggregation induced by AA, PAF, ADP, and epinephrine, while also examining its anti-inflammatory and analgesic potentials.

2. Materials and Methods

Sprague-Dawley rats weighing between 200 and 270 grams and mice weighing between 20 and 30 grams of both sexes were supplied by the animal house facility at Kohat University of Science and Technology, Kohat. All animal procedures adhered to the guidelines set by the Institute of Laboratory Animal Resources (NRC, 1996) and were assented to by the Ethical Committee of the Department of Pharmacy, Kohat University of Science and Technology, Kohat. The animals were kept in standard plastic cages with a 12-hour light and dark cycle and had non-restricted access to food and water. The chemicals used in the study included arachidonic acid, adenosine diphosphate, platelet-activating factor, epinephrine, acetic acid, and formalin.

2.1. Writhing Test

Adult male mice weighing 20–25 g (n =5) were used in this experiment, following the method described by Koster et al. (Koster 1959). The mice were randomly assigned to the following groups: Group I: vehicle-treated control group, Group II: pre-treated with diclofenac sodium (reference drug), and Groups III–V: pre-treated with three different doses of piperine. Thirty minutes after administering piperine or diclofenac sodium, the mice were injected intraperitoneally (i.p.) with 0.7% acetic acid (v/v) at a volume of 0.1 mL/10 g body weight. Each mouse was then placed individually in a glass beaker and observed for a 5-minute acclimatization period. Over the next 20 minutes, the number of abdominal writhes (characterized by stretching of the abdomen accompanied by the extension of a hind limb) was recorded. Control animals received normal saline (10 mL/kg, i.p.), while the reference drug, diclofenac sodium, was administered at a dose of 10 mg/kg, i.p.

2.2. Formalin Test

This test for pain assessment was conducted following the methodology outlined by Hunskaar and Hole (1987) (Hunskaar and Hole 1987). There were 5 mice in each group, weighing 20–25 g, and were injected 20 ul of 1% formalin in 0.9% saline subcutaneously into the dorsal hind paw, then put in a transparent observation box. Paw-licking behavior was

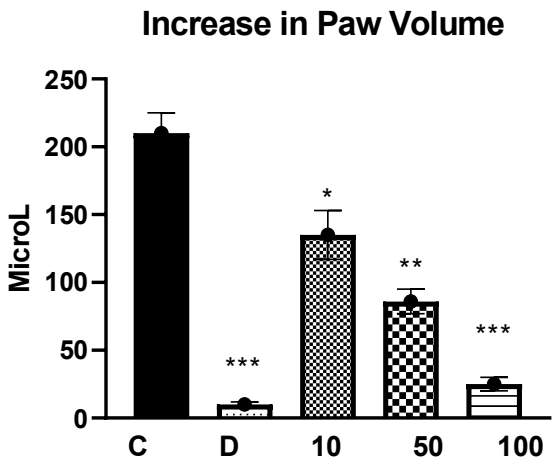

Figure 1. Effect of the Piperine (10, 50, and 100 µg/kg) and diclofenac sodium on carrageenan-induced paw edema in rats. Values represent mean ± SEM of 5 observations. *P<0.05, **P<0.01 and ***P<0.001, compared with control.

recorded as: Phase I (from 0 to 5 minutes) and Phase II (from 15 to 30 minutes) post-formalin administration. The time spent licking and biting the injected paw was noted in seconds. Mice were injected (i.p.) with different doses of piperine or diclofenac sodium (10 mg/kg i.p.), and control animals received a 0.1 mL/10g dose of the vehicle. The paw-licking time of the animals was compared to that of a control group and expressed as a percentage of inhibition.

2.3. Rat Paw Edema Assay

The carrageenan-induced hind paw edema test was conducted following the method described by Winter et al. (Winter, Risley, and Nuss 1962). Rats were randomly divided into groups of 5 animals each. A freshly prepared 1% carrageenan solution in distilled water (0.05 mL) was injected subcutaneously into the plantar surface of the hind paw to induce inflammation. Thirty minutes prior to the carrageenan injection, the rats were administered different doses of piperine or diclofenac sodium (20 mg/kg) intraperitoneally (i.p.). Control animals received an equivalent volume of the vehicle. Paw edema was measured using a volume displacement method with a plethysmometer (Ugo Basile 7150). Measurements were taken at baseline (before carrageenan injection) and at 1, 2, 3, and 4 hours after the injection. The difference in paw volume before and after the administration of the phlogistic agent (carrageenan) was used to determine the severity of edema. Additionally, the percentage inhibition of inflammation was calculated for each animal by comparing the results with those of the control group.

2.4. Anti-Platelet Aggregation Assay

The anti-platelet activity of the piperine extract was assessed using the method outlined previously (Hussain et al. 2010, Imran et al. 2012). Blood samples were collected from healthy volunteers who had not taken any medication for at least 7 days prior to the study. The blood was drawn via venipuncture and mixed with a 3.8% (w/v) sodium citrate solution in a 9:1 ratio. The mixture was then centrifuged at 260g for 15 minutes at 20°C to obtain platelet-rich plasma (PRP). The remaining blood was further centrifuged at 1,200g for 10 minutes to prepare platelet-poor plasma (PPP). Platelet

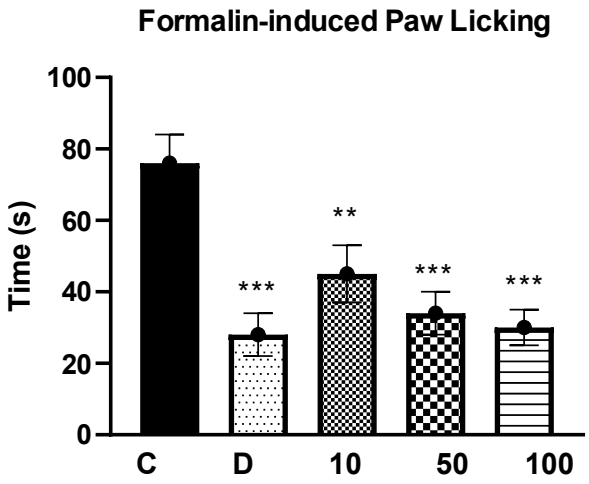

Figure 2: Effect of Piperine (10, 50, and 100 µg/kg) and diclofenac sodium on formalin-induced paw-licking time in mice. Values represent mean ± SEM of 5 observations. **P <0.01 and ***P<0.001, compared with control.

aggregation studies were conducted at 37°C using a dual-channel Lumi Aggregometer. For each test, 450 µL of PRP was pre-incubated with varying concentrations of piperine for 1 minute before being exposed to aggregating agents such as arachidonic acid (AA), adenosine diphosphate (ADP), platelet-activating factor (PAF), and epinephrine. The extent of aggregation was measured and compared to the control, recorded 4–5 minutes after the challenge.

2.5. Statistical Analysis

The data are expressed as mean ± SEM (standard error of the mean). Comparisons between experimental and control groups were performed using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test for post-hoc analysis. A p-value of less than 0.05 ($p < 0.05$) or less was considered statistically significant.

3. Results

Piperine significantly reduced the increase in paw volume in rats. In the control group, an increase in paw volume was observed, which was significantly reduced by the use of diclofenac sodium (figure 1). Piperine, at all the doses employed (10, 50, and 100 µg/kg), significantly reduced the paw volume while the highest dose (100µg/kg) produced an effect comparable to the standard drug (diclofenac sodium).

Formalin-induced paw-licking time was also reduced by piperine. Diclofenac sodium was the most potent in reducing the paw-licking time compared to the control group. All the doses of piperine used (10, 50, and 100 µg/kg) caused a significant reduction in the paw licking time compared to the control group (Figure 2). However, a comparable effect to diclofenac was observed with the highest dose only.

In the control group of animals, acetic acid-induced writhing was significantly elevated, which was reduced by the standard drug diclofenac and by the three doses of piperine. The most potent effect was observed with the dose of 100 µg/kg, which reduced the writhing even more than the reference drug. The lower doses of piperine also produced a significant effect compared to the control group (Figure 3).

Piperine inhibited the platelet aggregation induced by AA at all doses (1, 5, and 10 µM) but

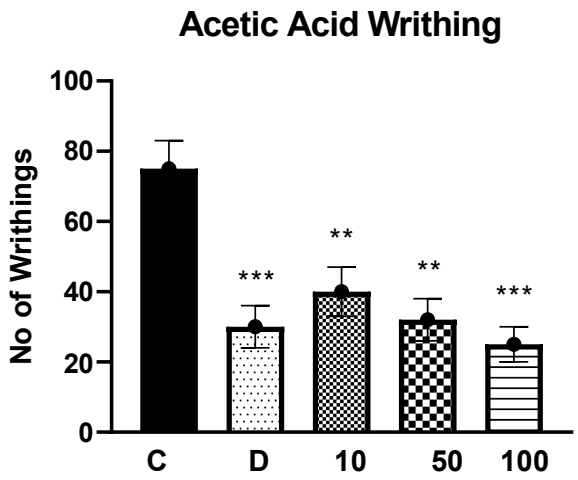

Figure 3: Effect of the Piperine (10, 50, and 100 µg/kg) on acetic acid-induced writhing in mice. Values represent mean ± SEM of 5–6 observations. **P<0.01 and ***P<0.001, compared with control.

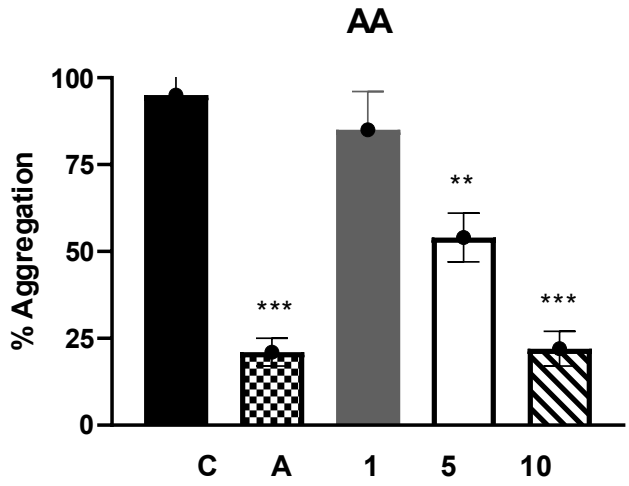

Figure 4: Inhibitory effect of Piperine (1, 5, and 10 µM) on AA-induced platelet aggregation. Values represent mean ± SEM of the % aggregation of the control maximum (n = 5). **P<0.01 and ***P<0.001, compared with the control.

the most pronounced effect was observed at 10 µM. Although all doses produced significant inhibition compared to the control, but 10 µM dose reduced platelet aggregation by around 75%, which was comparable to the effect of Aspirin (figure 4).

Piperine also inhibited the platelet aggregation induced by ADP at all doses (1, 5, and 10 µM), but the effect produced by 1 and 5µM doses was not statistically significant compared to the control effect. The most potent effect was observed at 10 µM, which was statistically significant compared to the control. However, the inhibition produced by a 10 µM dose was slightly less potent compared to the effect of Aspirin (Figure 5).

Piperine inhibited the platelet aggregation induced by PAF at all doses (1, 5, and 10 µM), but the effect was not statistically significant compared to the control. The most potent effect

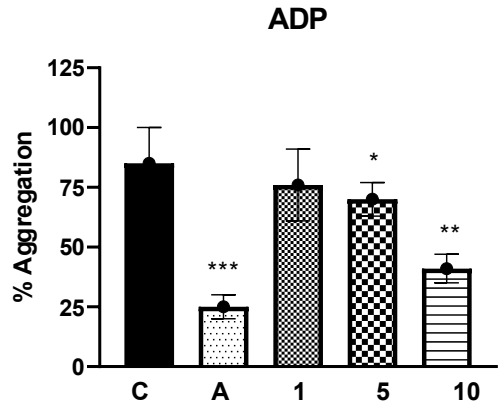

Figure 5: Inhibitory effect of Piperine (10, 50, and 100 µM) on ADP-induced platelet aggregation. Values represent mean ± SEM of the % aggregation of the control maximum (n =5). *P<0.05, **P<0.01 and ***P<0.001, significantly different compared with control.

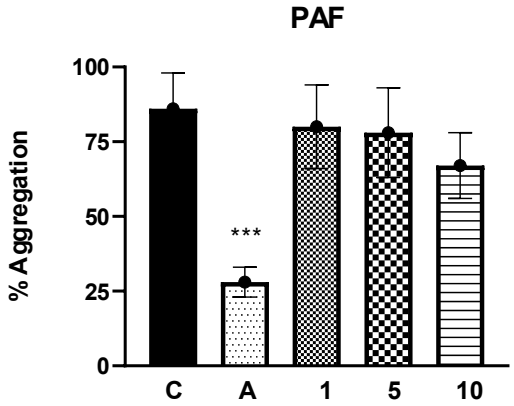

Figure 6: Inhibitory effect of Piperine (10, 50, and 100 µM) on PAF-induced platelet aggregation. Values represent mean ± SEM of the % aggregation of the control maximum (n = 5). ***P<0.001, significantly different compared with control.

was observed with Aspirin, which decreased the aggregation of platelets to around 25%. The 10 µM dose was the most potent of the piperine doses, but even that could not achieve a statistically significant reduction in platelet aggregation compared to the control (Figure 6). Piperine did not inhibit the platelet aggregation induced by epinephrine at any of the doses (1,5, and 10 µM). However, the standard drug (Aspirin) did produce significant inhibition of the epinephrine-induced platelet aggregation. Aspirin caused more than 50% inhibition of epinephrine-induced platelet aggregation (Figure 7).

4. Discussion

For the anti-nociceptive study, widely used pain models such as acetic acid-induced writhing and formalin-induced licking tests were employed. Acetic acid produces nociception by increasing the level of prostaglandins, serotonin, and histamine in peritoneal fluids, and this animal model is commonly used for screening peripheral analgesics. In the present investigation, the piperine inhibited acetic acid-induced writhing in mice, similar to diclofenac sodium, suggesting that the analgesic activity of the piperine might be related to the inhibition of the prostaglandin function (Ferreira 1972).

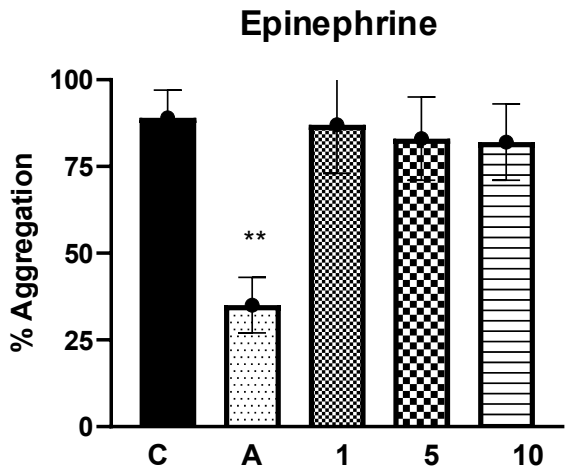

Figure 7: Inhibitory effect of Piperine (10, 50, and 100 µg/kg) on Epinephrine-induced platelet aggregation. Values represent mean ± SEM of the % aggregation of the control maximum (n = 5). **P < 0.01 significantly different compared with control.

Analgesic drugs such as diclofenac and aspirin, along with traditionally used medicinal plants like *Asparagus pubescens* and *Quassia amara* (Okpo, Fatokun, and Adeyemi 2001, Toma et al. 2003)have shown comparable effects in the acetic acid-induced writhing model. Acetic acid-induced constriction is considered to be a non-selective model because it triggers the release of endogenous mediators (prostaglandins) that can activate both peripheral nociceptors and neuronal responsive to NSAIDs, opioids, and other centrally acting medications (Vaz, Yunes, and Calixto 1996).

To get a better picture of the mechanism behind the analgesic action, the effect of piperine was also studied using the formalin-induced pain model. The formalin test is a widely recognized and reliable method for investigating the mechanisms of pain and evaluating analgesic compounds (Tjølsen et al. 1992). Formalin-induced pain consists of two phases: the first phase (neurogenic phase) results from the direct stimulation of sensory nerve fibers by formalin, while the second (inflammatory phase) is due to the release of inflammatory mediators such as histamine, serotonin, prostaglandins, and bradykinin (Hunskaar and Hole 1987). In this study, piperine pretreatment significantly reduced paw-licking responses in both phases of the formalin test, indicating a broad analgesic effect. Centrally acting drugs, such as morphine (opioids), suppress both phases of the formalin test, while peripherally acting drugs, like NSAIDs, predominantly target the latter phase (Santos et al. 1994, Shibata et al. 1989). Piperine's ability to reduce responses in both phases suggests that its analgesic effect involves central mechanisms.

Predominantly, piperine's analgesic effect was observed in the second phase of the formalin test, which suggests peripheral anti-inflammatory activity. Drugs like aspirin and phenylbutazone, are known to act peripherally (Shibata et al. 1989), provide pain relief by inhibiting COX in AA pathways (Levine 1994). The effects observed in chemical-induced pain models support the idea that piperine behaves similarly to NSAIDs.

Based on these results, the anti-inflammatory properties of piperine were further evaluated in

the carrageenan-induced rat paw edema model. Piperine showed significant anti-inflammatory effects like diclofenac sodium, a known reference compound. Carrageenan-induced acute inflammation progresses in two distinct phases: the early phase, characterized by edema mediated by histamine and serotonin, and the later phase, where vascular permeability is sustained by bradykinin and prostaglandins (Di Rosa, Giroud, and Willoughby 1971, Burch and DeHaas 1990). The second phase is especially responsive to potent anti-inflammatory agents and is frequently utilized to evaluate the anti-inflammatory properties of natural products (Della Loggia et al. 1986, Saeed et al. 1995). In this study, piperine showed predominant anti-inflammatory effects during the later phase of carrageenan-induced edema, suggesting that its action may be mediated by inhibition of prostaglandin activity. Similarly, diclofenac sodium also showed significant anti-edematous effects in this model. NSAIDs, such as diclofenac sodium, alleviate inflammation and swelling by blocking the synthesis of prostaglandins (Skoutakis et al. 1988). Evidence suggests that piperine may inhibit cyclooxygenase enzymes, which are involved in the production of prostaglandins and contribute to carrageenan-induced inflammation (Selvam and Jachak 2004).

Furthermore, COX inhibitors have exhibited the ability to suppress the aggregation of platelets (Siess, Cuatrecasas, and Lapetina 1983). Anti-inflammatory compounds were proven to be efficacious for the anti-platelet aggregation effect (Saeed et al. 1995, Jose, Ajith, and Janardhanan 2004). In other studies, piperine inhibited AA-induced platelet aggregation, further cementing its anti-inflammatory potential. Similar studies also suggest that piperine inhibits platelet aggregation via $cPLA_2$ and TXA_2 synthase attenuation rather than through COX-1 inhibition (Son et al. 2014). In addition to inhibiting AA-induced platelet aggregation, piperine was also potent in blocking ADP-induced platelet aggregation. However, it was relatively inactive against PAF and epinephrine-induced platelet aggregations.

In conclusion, piperine demonstrates promise against inflammation, pain, and platelet aggregation. Findings from the current study support the NSAID-like quality of piperine activity. The present data pose piperine as a novel source for the exploration and development of anti-inflammatory drugs.

Author Contributions

FA conceptualized the study, SMS and MA did the experimental work and data analysis, FA, SMS, and MA wrote the manuscript.

Acknowledgments

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Data Availability

All the data related to this study are available from the authors.

Study Approval

The research study was approved by the Department of Pharmacy, Kohat University of Science & Technology, Kohat, Pakistan.

Consent Forms

Consent forms were signed by the participants and are available from the authors.

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NA

Conflict Of Interest

The authors declare that they have no competing interests.

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