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Original Research Article

Drug-Piperaceae Herb Interaction Potency Through Analgesic, Anxiolytic, and Anti-Inflammatory Activity Studies

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ABSTRACT

Integrative therapy by combining conventional with herbal medicines is one of the choices for the community. However, there are opportunities for potential drug-herb interactions. Piperine, the primary alkaloid in the Piperaceae family, has been shown to change the pharmacokinetic profile of drugs and influence drug pharmacodynamics. This study aimed to determine the potential interactions between the combination of Piperaceae herb extract and conventional drugs based on their pharmacological activity *in vivo*. Black and Javanese long pepper extracts were prepared using the maceration method in ethanol. Then, the piperine level was determined using the TLC-densitometric method. Potential interactions were investigated through *in vivo* analgesic, anxiolytic, and anti-inflammatory activity studies. Piperine content in black and Javanese long pepper extract was 20.46% and 15.65%, respectively. Conventional drugs consumed with piperine or Piperaceae herbal extracts showed an increase in the latency time of mice on a hot-cold plate but a decrease in mice's survival time on the rotarod and oedema volume compared to single drug administration at various times. All combined treatments also enhance the percentage of analgesic (21.73% to 25.60%), anxiolytic (65% to ≥80%), and inflammation inhibitory (14.81% to ≥51.85%) activities of drugs. It concluded that piperine in black pepper or Javanese long pepper extracts has the potency to interact with drugs, especially with diclofenac sodium, alprazolam, and dexamethasone, based on the enhancement of their activity. Thus, further research is needed to determine its toxicity and pharmacokinetic profile to ensure the safety of these drug interactions.

***Keywords*:** Piperaceae, piperine, drug-herb interaction

Introduction

The rapid development and trend of natural-based medicine make integrative therapy, which combines conventional and herbal medicines, one of the choices for the community. However, there are opportunities for drug-herb interactions that need to be considered because they can be beneficial, detrimental, or even fatal.[1] Previous research found that 13.2% of people in Indonesia consumed combinations of herbal medicines with conventional medicines, with some of them having beneficial interactions (13.5%), harmful (18.9%), and no interaction (67.6%).[2]

This study focused on the interactions with Piperaceae herbs. This family is a natural vegetative plant with several species identified and spread across Meru Betiri Jember National Park.[3] The Piper genus, one of the Piperaceae family, is often used as spices, food mixtures, and traditional medicine with emollient, antirheumatic, diuretic, stimulant, abortive, anti-inflammatory, antibacterial, and antifungal properties.[4] The fruit contains piperine alkaloids known to have many properties, including anti-inflammatory and anti-arthritis agents, hypoallergenic, cardiovascular protection, anti-hyperlipidemia, anti-obesity, neuroprotective, and antiparkinson effects.[5]

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Previous studies have shown that piperine is a bioenhancer that can increase drug levels in the blood.[6] Piperine inhibits the activity of p-glycoprotein and drug-metabolising enzymes. It can increase drug absorption and bioavailability.[7] Other studies have shown that piperine can improve the pharmacokinetic profile of low bioavailability drugs, such as propranolol[8] and curcumin.[9] Treatment with piperine can also change the pharmacokinetic profile of fexofenadine[10], almotriptan[11], and other drug substrates of p-glycoprotein and cytochrome P450 enzymes. Piperine, a significant component of the genus Piper, is implicated in drug-herb interactions. So far, the existing research has only studied the interaction between piperine and drugs, while studies of Piperaceae herbs, when combined with drugs, still need to be evaluated. In fact, in society, the use of Piperaceae herbal extracts is more popular than a single preparation of piperine. People often use a combination of conventional medicines with herbal preparations in the hope of increasing the effects of drugs. For this reason, this study aimed to determine the potential for interactions between several Piperaceae herbal extracts when used together with drugs based on their *in vivo* pharmacological activity.

Materials and Methods

Materials

The sample materials were black pepper (*Piper nigrum* L.) powder, purchased from Materia Medica, Malang, and Javanese long pepper (*Piper retrofractum* Vahl.) obtained from Kalisat, Jember Regency. The samples were collected in August 2022 and were determined by Materia Medica Batu with the certificate number 074/599/102.20-A/2022. Piperine (Sigma-Aldrich), alprazolam (Mersi), diclofenac sodium (First Medipharma), and dexamethasone (Novapharin) tablets were also prepared. The chemicals used include 95% ethanol, hexane, ethyl acetate, aqua-dest, and sodium-CMC. Other consumables are capillary

tubes and TLC plates. The tools used were Rotarod (Ugo Basile), hot-cold plate (Ugo Basile), plethysmometer (Ugo Basile), TLC-Densitometer, UV lamp, analytical balance (Ohaus), animal scales (Ohaus), and rat sonde.

Ethical approval and experimental animals
Ethical approval was obtained from the ethics committee of FKG-University of Jember, and a certificate of ethical approval number 1637/UN25.8/KEPK/DL/2022 was issued. The healthy Balb-c male mice, 6-8 weeks old and weighing 150-200 g body weight, were obtained. The animals were housed in plastic cages with free access to a normal diet and water. The rats were kept in a temperature-controlled room (22 ± 2°C), with a 12 h light/dark cycle and relative humidity of 55 ± 10%. They were acclimatised to the laboratory conditions for one week before the experiments. The ethics committee of FKG-University of Jember approved all experimental procedures. Ninety-six animals were used for the experiments.

Preparation and Extraction of plant materials
The Javanese long pepper was dried at room temperature, away from direct sunlight, for several days, then sorted and powdered. Previously, the Black pepper sample was purchased in powdered form. About 150 g of each Piperaceae powder was soaked with 1.5 L of 95% ethanol (1:10) with occasional stirring and then filtered. The marc was re-extracted two times. The filtrate was evaporated using a rotary evaporator at 40°C and then dried in an oven at 50°C until a dry extract was obtained, coded BPE for Black pepper extract and JLPE for Javanese long pepper extract.

Determination of Piperine Levels
Each piperaceae extract was prepared at a 200 µg/mL concentration in 95% ethanol (p.a), whereas a standard piperine solution was prepared at 100 µg/mL. Analysis was performed using TLC-densitometry with 60 F254 silica gel TLC plates as a stationary phase and hexane-ethyl acetate (1:1) as a mobile phase. 3 µL sample and standard piperine were spotted, then eluted with mobile phase until the spot appeared and was observed under a UV lamp λ 254 nm.

Drug–herbs interaction potency study through in vivo activity test
Thirty-two mice divided into eight groups consisting of negative control and seven treatment groups: (1) piperine (PIP) 20 mg/kg BW, (2) medicines (sodium diclofenac (DIC) 10 mg/kg BW or alprazolam (ALP) 1 mg/kg BW or dexamethasone (DEX) 3.2 mg/kg BW, (3) black pepper extract (BPE) 100 mg/kg BW, (4) Javanese long pepper extract (JLPE) 125 mg/kg BW, (5) DIC/ALP/DEX+PIP, (6) DIC/ALP/DEX+BPE, (7) DIC/ALP/DEX+JLPE.

Analgesic Activity Test
The test animals were placed on a hotplate at 55 ± 1ºC[12] to measure their basal latency time. Mice latency time not exceeding 15 seconds was used for the study, and treatment was given according to each group. The time between the mice being placed and the mice giving a response (licking or lifting their feet or jumping) was calculated as the latency time. The cut-off set was 15 seconds to prevent tissue damage. Latency time was measured periodically at 0, 0.5, 1, 1.5, 2, and 3 hours. The percentage of analgesic activity was determined at a peak time.[13]

Anxiolytic Activity Test
Motor coordination and balance were tested using mice Rotarod. Before testing, the mice were trained to stay on the Rotarod for at least 60 seconds a few days before regularly. The mice were placed on metal parts and ran at 40 rpm/minute. The time mice maintained balance by walking on a spin was measured periodically at 0 (basal), 60, 90, 120, and 180 minutes. At the peak time, the percentage of anxiolytic activity was calculated.[14]

Anti-inflammatory activity test
The mice's plantar basal volume was measured by dipping the sole in the plethysmometer. Mice were injected with 0.1 mL carrageenan intraplantar. Carrageenan 1% was prepared by mixing 100 mg powder in 1% sodium CMC suspension. Then, the plantar volume was measured at 0, 30, 60, 90, 120, 150, and 180 minutes after being treated. The oedema volume was calculated using the plantar volume at a specific time after carrageenan injection minus the volume before carrageenan injection. Then, each treatment's percentage of inflammation inhibitory activities was calculated after 3 hours.

Statistical analysis
The mice's oedema volume, latency, and endurance time were analysed with One Way ANOVA or Kruskal Wallis test with a confidence level of 95 %, followed by a post hoc test to determine differences between groups.

Results and Discussion

The extracts' yield obtained in this experiment was greatly improved by repeated maceration. The BPE and JLPE extract yields were 11.7% and 10.5%, respectively. This result still meets the requirements contained in the Herbal Pharmacopeia, which is 11.3% for black pepper and 8.3% for Javanese long pepper ethanol extract.
The piperine level in the extracts was determined by the standard regression equation y=241.9+9.445x. The result of the extract resolution using TLC plates is shown in Figure 1. The results showed PIP, BPE, and JLPE spots with the same RF value (0.64). The results showed the percentage of piperine content in BPE and JLPE, respectively, to be 20.46 ± 2.92 % and 15.65 ± 1.69 %.
Herbal Pharmacopoeia (2017) states that thick black pepper extract contains not less than 48.60% piperine, and in Javanese chilli extract, not less than 4.40%. Based on these references, the piperine content in BPE is much lower than the standard, whereas it is higher in JLPE. It may be due to various factors, including differences in growing places, which allow variations in their phytochemical components. The level of piperine found in BPE was higher than in JLPE. This was in line with the previous studies that showed that the highest piperine content of the entire piper genus was found in *Piper nigrum* L, with a range of 2-9%[6], followed by *Piper colubrinum* (4.9%), *Piper longum* (3.7%), *Piper retrofractum* (2.3%), *Piper cubeba* (1.2%), and *Piper betle* (0.9%).[4]
The study also evaluated the *in vivo* effect of the drug–herbs interaction on the potency of analgesic activity of conventional medications. The result of the combination of Piperaceae herbs with diclofenac is shown in Figure 2. At 30 minutes post-treatment, the latency time of mice in all groups decreased, starting optimally at the first hour and reaching its peak at 1.5 hours, except for the BPE and JLPE, which peaked in the first hour. Studies have shown that the onset of diclofenac sodium is achieved within 60 minutes.[15] Statistically, a significant difference in latency was only found at 60 and 90 minutes. According to the data in Table 1, at 60 minutes, the treatment group with DIC, both alone and in combination, began to show a similar effect marked by an increase in latency time. Meanwhile, the piperine and extract groups did not show a significant difference from the control, and no activity in 60 or 90 minutes was observed.

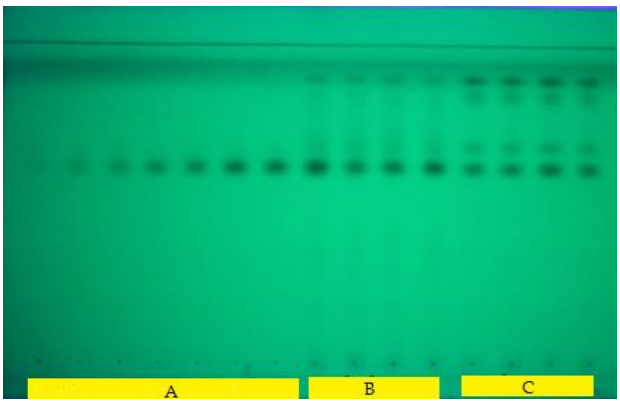

Figure 1: Results of piperine qualitative analysis using the TLC method. Piperine standart (A), BPE (B), JLPE (C).

The treatment with single DIC was not statistically significantly different from all combined DICs based on latency time. It was inconsistent with the existing theory that piperine has a pharmacokinetic interaction with drugs that are substrates of the drug-metabolising enzymes. Diclofenac sodium is absorbed fast through the GI tract, distributed, and eliminated quickly.[16] In the body, diclofenac sodium is mainly converted to 4-hydroxy diclofenac by CYP2C9. Other metabolites, such as 5-hydroxy diclofenac and 3-hydroxy diclofenac, were mediated by CYP3A4.[17] CYP2C9 is the primary enzyme responsible for NSAID metabolism, including diclofenac sodium.[18] Meanwhile, the CYP3A4 enzyme is responsible for significant first-pass metabolism in diclofenac, causing low bioavailability of diclofenac (only about 60% reaches the systemic circulation).[19] Piperine is known to inhibit CYP3A4 in humans[20] and is a selective competitive inhibitor of this enzyme, with an IC_{50} value of <10 µM. In addition, piperine also has potent inhibitory activity on CYP2C9 with an IC_{50} value < 100 µM.[21] The metabolism of diclofenac, a substrate of CYP2C9 and CYP3A4 enzymes, can be inhibited, causing an increase in drug levels in the blood, which can also affect its effectiveness. Other *in vivo* studies have shown that combining piperine with glimepiride[22] and warfarin[23] can change pharmacokinetic parameters and increase activity through CYP2C9 inhibition by piperine. A clinical study was conducted to determine piperine's effect on diclofenac's pharmacokinetics in healthy subjects. These studies showed that treatment with piperine could significantly increase Cmax, AUC, and t1/2 but decrease Kel and diclofenac clearance by inhibiting CYP2C9 enzymes.[24]

In this study, the pharmacokinetic interactions that occurred were not followed by biological relevance, so an increase in diclofenac levels did not change the drug's activity. It occurs because diclofenac sodium metabolism does not only involve two enzymes (CYP2C9 and CYP3A4), but other metabolising enzymes are implicated. *In vitro* studies have shown that CYP2C8 also catalysed the 4-hydroxylation process. Diclofenac is converted to diclofenac glucuronide by UGT2B7, then to 4-hydroxy diclofenac glucuronide by CYP2C8, to be excreted in the urine and faeces. It explains the possibility of other pathways for eliminating diclofenac even though CYP2C9 and CYP3A4 have been inhibited.[17]

From Table 2, only the administration of BPE combined with DIC enhanced the effect of DIC. However, Piperaceae extract has complex constituents that can influence the effect. The variation in piperine levels coupled with the various phytochemical components contained in these plant extracts can also affect the effects, which can be seen from the differences in the latency time values and percentage of analgesic activity for each group.

The anxiolytic effects of the extracts are shown in Figure 3. The mice's endurance time decreased at 60 minutes, tended to last until 90 minutes, and increased at 120 and 180 minutes. The result was consistent with data on the onset of alprazolam, achieved within 20-60 minutes, with peak plasma concentrations reaching 1-2 hours. The survival time of mice on the Rotarod showed significant differences at each measurement time, starting from 60, 90, 120, and 180 minutes, based on the Kruskall-Wallis test (Table 3). At 60 minutes, there was a significant difference between the control and all treatment groups except JLPE. Likewise, in subsequent times, JLPE also did not significantly differ from the control group, which means that JLPE was the only extract that did not show anxiolytic activity. The group that was treated with ALP and BPE had similar profiles, which started to show anxiolytic effects at 60 minutes. However, as the average endurance time indicated, this activity decreased over time, similar to the control group at 120 minutes. Interestingly, it was found that the combination treatment had a significantly different endurance time and was much lower than the single administration of ALP, especially at 60 and 90 minutes. From Table 4, the anxiolytic activities of ALP combined with PIP, BPE, or JLPE were also higher than a single administration of ALP. Based on these results, PIP, BPE, and JLPE exhibited anxiolytic activity. In line with previous research, piperine has anxiolytic activity in stressed and non-stressed mice by inhibiting neuronal NOS.

Table 1: The mice's latency time at 60 and 90 minutes after various treatments

Groups	Latency time (second)	
	60*	90#
Control	6.4 ± 0.19[a]	6.6 ± 0.29[a]
DIC	7.8 ± 0.27[ab]	8.4 ± 0.14[b]
PIP	5.8 ± 0.39[ac]	6.4 ± 0.63[a]
BPE	6.0 ± 0.21[ac]	5.9 ± 0.31[a]
JLPE	7.6 ± 0.79[ab]	7.1 ± 0.79[a]
DIC + PIP	7.6 ± 0.45[ab]	8.3 ± 0.66[b]
DIC + BPE	8.1 ± 0.82[b]	8.8 ± 0.56[b]
DIC + JLPE	7.6 ± 0.28[ab]	7.9 ± 0.17[b]

Results are expressed as the mean ± SEM (n=4 animals per group). The same superscript means no significant difference. $p<0.05$ were considered significant based on one-way ANOVA (*), followed by LSD and Kruskall-Wallis, followed by the Mann-Whitney (#) test.

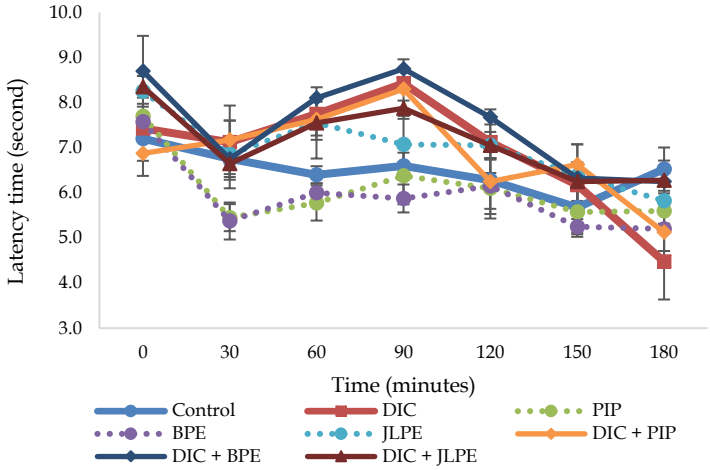

Figure 2: Effects of various treatments on mice latency time. Results are expressed as the mean ± SEM (n=4 animals per group). $p<0.05$ were considered significant based on one-way ANOVA (*) and the Kruskall-Wallis test.

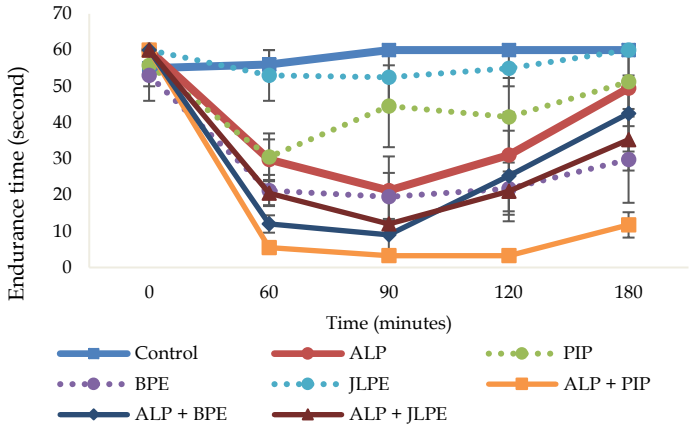

Figure 3: Effects of various treatments on mice endurance time. Results are expressed as the mean ± SEM (n=4 animals per group). $p<0.05$ were considered significant based on the Kruskall-Wallis test (*).

However, it only increased GABA levels in mice without stress.[25] Administration of pepper fruit methanol extract to Alzheimer's mice model showed anxiolytic activity through the Elevated Plus-Maze (EPM) [26] and forced swimming test,[27] as well as the essential pepper oil. It also exhibited anxiolytic activity through the involvement of 5-HT1A receptors.[28] The combination with PIP, BPE, or JLPE also enhanced the activity of alprazolam. Despite the suggestion that piperine and BPE are known to have similar activity as previously described, this finding may also be related to the presence of pharmacokinetic interactions between piperine and alprazolam, especially in the metabolic phase. Alprazolam is converted into two primary metabolites, namely 4-hydroxy alprazolam and α-hydroxy alprazolam, through CYP3A4 enzymes.[29] This enzyme inhibition increases alprazolam blood levels and enhances its effect. However, there has been no research on the interaction between alprazolam and piperine. Other similar studies have proven a pharmacokinetic interaction between piperine and other drugs as substrates of CYP3A4, including midazolam[30] and carbamazepine.[31] Based on these studies, piperine can increase bioavailability and half-life but decrease the constant clearance and elimination of the two drugs. The increased effect of ALP due to the interaction with PIP was also shown in combination with BPE and JLPE. The possible reason could be that piperine, the main phytochemical compound in the Piperaceae family, occurs in a large amount in black pepper and Javanese long pepper.[6] However, the other phytochemicals in these two plants could also influence their activity, although this may not be as significant as the combination with piperine.

The results of anti-inflammation activity are shown in Figure 4 and Table 5. As observed, the combination treatment of DEX+PIP/BPE/JLPE reduced the oedema volume of carrageenan-induced mice better than dexamethasone alone, especially at 150 and 180 minutes. The body metabolises dexamethasone to 6α-hydroxy dexamethasone and 6β-hydroxy dexamethasone by the CYP3A4 enzyme.[32] As previously explained, the inhibition of this enzyme by piperine produces an increase in dexamethasone levels in the blood, so the effect will also increase. In line with this theory, Table 6 shows that combining PIP, BPE, or JLPE with ALP can increase the inflammation inhibitory activity. Interestingly, this study showed a change in onset from 60 to 30 minutes in the combined DEX+PIP/BPE/JLPE treatments. Reports have shown that dexamethasone reaches peak blood levels within 1 hour.[33] The shift in Tmax is presumable not only due to the inhibition of metabolising enzymes but also due to the inhibition of the P-glycoprotein (Pgp) by piperine. Pgp is a member of the ATP binding cassette (ABC) transporter superfamily, which plays a role in drug transport. In the intestine, Pgp pumps the drug back into the lumen, which causes a decrease in absorption. Apart from enterocytes, Pgp is also expressed in hepatocytes, contributing to first-pass metabolism.[34] Piperine had vigorous Pgp inhibitory activity on the transport of several drugs *in vitro* with an IC_{50} value < 100 µM. Pgp inhibition has been shown to affect the pharmacokinetics of several drugs, such as fexofenadine[10] and domperidone,[11] substrates of Pgp. Dexamethasone itself is one of the physiological substrates of PgP.[35] Inhibition of these transporters by piperine can increase the bioavailability and absorption of the drug. This explanation may cause a shift in the Tmax of DEX when given together with PIP or Piperaceae extracts, as shown in this study.

Table 2: Percentage of analgesic activity after various treatments

Groups	Analgesic activitiy (%)
DIC	21.73
PIP	-2.68
BPE	-8.63
JLPE	5.65
DIC+PIP	20.24
DIC+BPE	25.60
DIC+JLPE	15.18

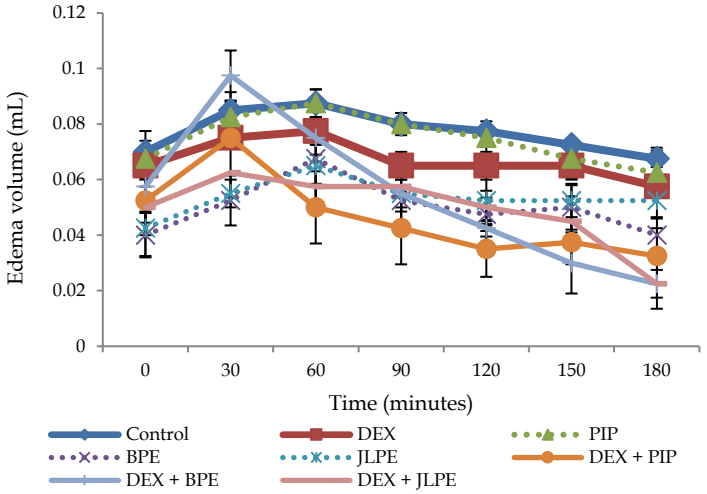

Figure 4: Effects of various treatments on mice oedema volume per unit time. Results are expressed as the mean ± SEM (n=4 animals per group). $p<0.05$ were considered significant based on the Kruskall-Wallis test (*).

Table 3: The mice's endurance time on Rotarod at 60, 90, 120, and 180 minutes after various treatments

	Endurance time (second)			
Groups	**60**	**90**	**120**	**180**
Control	56 ± 4.0^{a}	60 ± 0.0^{a}	60 ± 0^{a}	60 ± 0.0^{a}
ALP	30 ± 5.6^{b}	21 ± 9.4^{b}	31 ± 11.8^{b}	50 ± 10.5^{a}
PIP	31 ± 6.5^{b}	45 ± 11.3^{ab}	42 ± 10.8^{ab}	51 ± 8.8^{a}
BPE	21 ± 4.3^{b}	20 ± 6.6^{b}	22 ± 7.2^{b}	30 ± 11.9^{ab}
JLPE	53 ± 7.0^{a}	53 ± 7.5^{a}	55 ± 5.0^{ab}	60 ± 0.0^{a}
ALP+PIP	6 ± 1.5^{c}	3 ± 1^{c}	3 ± 0.8^{cb}	12 ± 3.5^{b}
ALP+BPE	12 ± 2.4^{cd}	9 ± 3.9^{bc}	25 ± 12.5^{b}	43 ± 10.5^{a}
ALP+JLPE	21 ± 3.3^{bd}	12 ± 1.4^{bc}	21 ± 5.5^{b}	35 ± 8.5^{ab}

Results are expressed as the mean ± SEM (n=4 animals per group). The same superscript showed no significant difference. p<0.05 were considered significant based on Kruskall-Wallis, followed by the Mann-Whitney test.

Table 4: Percentage of anxiolytic activity after various treatments

Groups	Anxiolytic activity (%)
ALP	65.00
PIP	25.00
BPE	66.67
JLPE	11.67
ALP+PIP	95.00
ALP+BPE	85.00
ALP+JLPE	80.00

Conclusion

This study concluded that black pepper or Javanese long pepper extract containing piperine or piperine alone has potential drug interactions, especially with diclofenac sodium, alprazolam, and dexamethasone. The study results highlighted that these interactions may be beneficial because they can increase the drug's pharmacological activity. Further research is needed for its toxicity and pharmacokinetic profile to ensure the safety of these drug interactions. The results of this study provide a formal reference for persons who may use a combination of Piperaceae herbal preparations and conventional medicines.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 5: The mice's oedema volume at 30, 90, 150, and 180 minutes after various treatments

	Edema volume (mL)			
Groups	**30**	**90**	**150**	**180**
Control	0.085 ± 0.003^{a}	0.080 ± 0.000^{a}	0.073 ± 0.002^{a}	0.068 ± 0.003^{a}
DEX	0.075 ± 0.003^{a}	0.065 ± 0.005^{b}	0.065 ± 0.005^{ac}	0.058 ± 0.002^{b}
PIP	0.083 ± 0.009^{a}	0.080 ± 0.004^{a}	0.068 ± 0.007^{ac}	0.063 ± 0.009^{a}
BPE	0.053 ± 0.009^{b}	0.053 ± 0.009^{b}	0.050 ± 0.008^{b}	0.040 ± 0.006^{a}
JLPE	0.055 ± 0.005^{b}	0.055 ± 0.013^{a}	0.053 ± 0.006^{b}	0.053 ± 0.006^{bc}
DEX+PIP	0.075 ± 0.003^{c}	0.043 ± 0.013^{b}	0.038 ± 0.008^{b}	0.033 ± 0.010^{d}
DEX+BPE	0.098 ± 0.009^{d}	0.055 ± 0.005^{b}	0.030 ± 0.011^{b}	0.023 ± 0.009^{d}
DEX+JLPE	0.063 ± 0.009^{cd}	0.058 ± 0.009^{b}	0.045 ± 0.009^{bc}	0.023 ± 0.005^{d}

Results are expressed as the mean ± SEM (n=4 animals per group). The same superscript showed no significant difference. $p<0.05$ were considered significant based on Kruskall-Wallis, followed by the Mann-Whitney test.

Table 6: The percentage of inflammation inhibitory activity after various treatments

Groups	% inflammation inhibitory activity
DEX	14.81
PIP	7.41
BPE	40.74
JLPE	22.22
DEX+PIP	51.85
DEX+BPE	66.67
DEX+JLPE	66.67

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