Isolation, Chemical Modification and *In vivo* Pharmacological Screening of Piperine and its Derivatives

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ABSTRACT: Piperine (**1**) extracted from black pepper was transformed into its derivatives piperic acid (**2**) and piperonal (**3**) with good yields (69-76%). Animal models were used to examine the compounds' *in vivo* peripheral as well as central analgesic and anti-inflammatory effects. In the peripheral analgesic experiment, compound **2** displayed remarkable action having writhing inhibition by 78% at 50 mg/kg dose, superior to that of **1** (74% writhing inhibition) when administered at the same dose and compared with standard diclofenac sodium (85%). Concerning central analgesic efficacy, piperonal outperformed compounds **1** and **2** at 25 mg/kg dose, with % tail-flick elongation times of 194%, 178%, and 178% at 30, 60, and 90 min, respectively. Conversely, at 50 mg/kg dose, piperic acid exhibited the highest activity, demonstrating % tail-flick elongation times of 231%, 213%, and 206% at 30, 60, and 90 min, respectively, as compared to the standard morphine (278%, 247%, and 160%) in the same duration. In anti-inflammatory property evaluation, piperonal portrayed outstanding effects with paw edema inhibitions of 57%, 66%, 76%, and 81%, respectively from 1st hour onwards, compared to standard aceclofenac (61%, 72%, 78%, and 89%) and parent compound piperine (20%, 34%, 51%, and 60%). This study suggests that piperine derivatives could act as promising leads for future drug development.

Key words: *Piper nigrum* L., piperine, piperine derivatives, peripheral and central analgesic activity, anti-inflammatory activity.

INTRODUCTION

Black pepper (*Piper nigrum* L.), a member of the piperaceae family of tropical plants, is a plenteous source of culinary spices and a diverse variety of biologically active compounds.[1] Despite being native to India, it is also seen to grow in Bangladesh among other southeast Asian regions, due to fulfilling the optimal warm and humid conditions for its growth. Owing to its dominance in the spice industry, black pepper has attracted worldwide attention because of its momentous promise as a source of new lead compounds with varied pharmacological attributes for future drug development. Piperine, one of the predominant bioactive components of black pepper, has been endowed with intriguing pharmacological attributes such as anticancer[2,3] and neuroendocrine modulator effects, according to mounting data from *in vitro* and *in vivo* investigations.[4] Its anti-inflammatory, antiulcer, antioxidant, expectorant, anti-flatulent and cholesterol-lowering properties have also been proven in several investigations.[5-8] Reportedly, black pepper contains 3-9% of piperine, 6% of pungent resin, 1-2.5% of volatile oil, 30% of piperidine and starch.[9] Additionally, lignans and flavonoids have also been found in this condiment.[10] Because most natural products are biosynthesized in only trace quantities and need time-consuming processes for isolation, their application as drugs or as a basis for semi-synthesis of newer therapeutic molecules is constrained. Nonetheless, considering its abundance and simplicity of isolation, piperine has an

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advantage over these restrictions.[11] Even though the medicinal applications of piperine date back to the dawn of time, the activity of many of its derivatives, however, is yet substantially unknown. Thus, appropriate modifications to the molecule and synthesis of its analogues with reduced side effects, maximized benefits as well as economic feasibility may present enhanced privileges, particularly in varied treatment modalities in the interest of public health.

Irrespective of the etiology, inflammation is typically an initial trigger for pain. As the involvement of the inflammatory cytokines unraveled, there is currently a comprehensive insight of the mechanisms by which numerous anti-inflammatory medications can reduce inflammation and alleviate pain. Non-steroidal anti-inflammatory drugs (NSAIDs) have been the principal choice for the treatment of pain and inflammation till date. Nevertheless, a number of studies have revealed that NSAIDs might hinder muscle regeneration and negatively impact kidney function.[12,13] Additionally, the main side effects of nonselective NSAIDs include serious gastrointestinal distress, gastritis, ulceration, bleeding and can even cause mortality.[13] As a consequence of considerable adverse effects associated with steroidal and NSAID drugs, there is growing interest in natural substances, including nutritional supplements and herbal therapies, which have been used for years to treat pain and inflammation. As piperine is a naturally occurring molecule and present in abundance in black pepper which is used frequently in our regular meals as a condiment, piperine derivatives might be potential drug candidates to develop safer drug molecules for managing pain and inflammation. Therefore, this study emphasizes on the synthesis of several structurally modified piperine molecules and ascertaining their biological effects, which can have novel therapeutic implications as analgesic and anti-inflammatory agents.

MATERIALS AND METHODS

Chemicals and equipment. The chemical reagents utilized in the procedure were acquired from Sigma-Aldrich, Fischer, Merck, Loba Chemie and several other reputed chemical suppliers. Analytical grade solvents including methanol, ethanol, diethyl ether, ethyl acetate, *n*-hexane, toluene, chloroform, tetrahydrofuran etc. were purchased from Merck (Germany). All reactions were conducted in glassware that had been thoroughly cleaned and dried. Before use, all of the solvents employed in the reactions were refluxed and distilled over calcium hydride (CaH_2) to make them super dried. The reaction progress was observed using thin layer chromatography (TLC) and UV light (254 nm) or a para-anisaldehyde/H_2SO_4/glacial acetic acid spray followed by heating on a hot plate over 120°C for around 1-2 min to detect TLC spots. A rotary evaporator was utilized for evaporating the solvents from the reaction mixture at reduced pressure (Heidolph, Germany). Column chromatography employing silica gel 60-120 mesh was used to decontaminate the crude substances. FTIR spectra with spectral range of 4000-700 cm^{-1} were obtained from the Centre for Advanced Research in Sciences (CARS), University of Dhaka, using an FTIR spectrophotometer (Model- IRPrestige 21, Shimadzu Corporation, Japan) with a resolution of 2 cm^{-1}. The ^1H NMR (400 MHz) spectra were acquired from Bangladesh Council of Scientific and Industrial Research (BCSIR) in deuterated chloroform ($CDCl_3$) using a Bruker 400 UltraShield™ NMR spectrometer. The internal reference standard used in this assay was tetramethylsilane (TMS). The chemical shift (δ) measurements have been expressed in parts per million (ppm), while spin-spin coupling constants (*J*) are represented in hertz (Hz).

Preparation of black pepper fruit powder. Dried black pepper fruits were procured from a regional market in Dhaka, Bangladesh. The fruits were washed and cleaned to make them free of stones and other inadmissible impurities. The sun-dried fruits were then pulverized to a fine particle size in an electrically powered grinder.

Extraction. The finely ground black pepper powder (100 g) was mixed with 500 ml distilled ethanol and refluxed for 3h at 80°C. After subsequent filtration of the mixture using a vacuum filter, the residual solvent was evaporated using a rotary evaporator under reduced pressure.[14]

Isolation, purification and recrystallization. The isolation, purification and recrystallization were accomplished by modification of a reported method.[14] Ten percent of ethanolic KOH solution (10 ml) was poured into the concentrated pepper extract and stirred it using a magnetic stirrer for 2 hours. The solution was filtered and the filtrate was collected where water was added gradually in a dropwise manner. A yellow precipitation was observed after allowing the mixture to sit overnight in a cool place. Finally, the solid precipitate was collected by filtering out the liquid and it was recrystallized with the modified solvent system *n*-hexane: DCM (2:1), acquiring 3 g of piperine in pure crystalline form. Identity of the compound was confirmed by comparing the spectral data with the reported values.[15]

Piperine (1). Yellow needle-like crystals; Rf = 0.78 (ethyl acetate: *n*-hexane = 2:1); FTIR (cm^{-1}): 2939.9 (C-H, aromatic), 2919.9 (C-H, aromatic), 2862.6 (-N-CH_2-), 1632.3 (-CO-N-), 1582.2 (C=O), 1490.7 (C=C, aromatic); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42-7.38 (m, 1H), 6.97 (dd, *J* = 8.6, 1.6 Hz, 1H), 6.89 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.79-6.71 (m, 3H), 6.43 (d, *J* =14.8 Hz, 1H), 5.98 (s, 2H), 3.59-3.52 (m, 4H), 1.68-1.56 (m, 6H); Yield: 3%.

Synthesis of piperine derivatives

Preparation of piperic acid (2) from piperine (1). Piperine (0.150 g) was taken in a 250 ml round-bottom flask and 9 ml of 20% ethanolic potassium hydroxide (KOH) was added to piperine (**1**). The mixture was refluxed for 12 h at 70°C and then cooled down to room temperature. The pH of the solution was adjusted to 3.0 by adding required amount of 1M HCl to it and a pH meter and litmus paper were used to verify the pH. To extract the crude product from the aqueous layer, dichloromethane (DCM) was used three times. After that, the organic layer was washed with brine solution and dried over anhydrous sodium sulfate (Na_2SO_4) for removing the undesirable water. A rotary evaporator was employed to evaporate the organic solvent completely. Pure crystals were obtained by recrystallizing the crude product with a methanol: water (8:2) mixture, which was further characterized using different spectroscopic methods as piperic acid (**2**).

Piperic acid (2). Pale green crystals; R_f = 0.75 (ethyl acetate: *n*-hexane = 2:1); FTIR (cm^{-1}): 1675.2 (carboxylic functional group), 1626.6 (C=C stretch), 1606.6 (conjugated C=C), 1563.6 (acidic C=O), and 3000-2500 (C-H, aromatic); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.469 (m, 1H), 6.99 (d, *J* = 1.6 Hz, 1H), 6.94 (dd, *J* = 8, 1.6 Hz, 1H), 6.79 (m, 3H), 6.48 (d, *J* = 14.8 Hz, 1H), 5.982 (s, 2H); Yield: 76%.

Synthesis of piperonal (3) from piperine (1). A solution of piperine (**1**, 0.15 g in 8 ml THF) was added in drops over the course of 4 hours to an aqueous solution of $KMnO_4$ (0.42 g in 30 ml distilled water). The mixture was stirred for the next 4 hours using a magnetic stirrer at room temperature after the $KMnO_4$ solution was added completely. When the reaction was over, resultant MnO_2 precipitate was filtered off, collecting the straw-colored filtrate. Extraction of the crude product was done using diethyl ether (3x30 ml). Brine solution was used to wash the organic extract which was consequently dried over anhydrous sodium sulfate (Na_2SO_4). Under reduced pressure, diethyl ether was evaporated completely, yielding a dark yellowish-orange crude oil that solidified when cooled. This concentrated product was purified by column chromatography (*n*-hexane: ethyl acetate = 3:1), which yielded a sweet-smelling, yellowish-orange clear oil, which was characterized using various spectroscopic methods as piperonal (**3**).

Piperonal (3). Yellowish-orange oil; R_f = 0.81 (ethyl acetate: *n*-hexane = 3:1); FTIR (cm^{-1}): 1682.4 (stretching of polar C=O, most intensive signal), 1600.8 (conjugated C=C), 1444.9 (aromatic ring stretch), 743.9 (C-H, aromatic); ^{1}H NMR (400 MHz,

$CDCl_3$) δ: 9.87 (s, 1H), 7.47 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.39 (d, *J* = 1.6 Hz, 1H), 6.99 (d, *J* = 8.0 Hz, 1H), 6.13 (s, 2H); Yield: 69%.

Pharmacological screening. All the synthesized derivatives, along with piperine, were examined through different *in vivo* tests, where their analgesic and anti-inflammatory functions were determined in animals exposed to heat and chemical inducers. Ethical permission was obtained from Ethical Review Committee of Faculty of Biological Sciences (Ref. number. 187/Biol. Scs.), University of Dhaka.

Experimental animal. Swiss albino mice (*Mus musculus)* and Wistar rats (*Rattus norvegicus*) of both sexes, weighing 25-30 g and 140-150 g, respectively, and 4-5 weeks of age, were acquired from the animal-house of Jahangirnagar University. The animals were placed in polypropylene boxes (30cm×20cm×13cm) for 7 days before the experiment, in the animal-house facility of the Institute of Nutrition and Food Science (INFS), University of Dhaka, under regular environmental conditions (22 ± 1°C with 12-hour light-dark cycle). The animals received rodent food and water as suggested by icddr,b (International Centre for Diarrhoeal Disease Research, Bangladesh). For 7 days prior to the test, these animals were kept in the location where the experiment would take place since they are extremely sensitive to environmental changes. The mice were only given water 12 hours before the trial. The Swiss Academy of Medical Sciences' ethical norms and recommendations were followed, and the fewest animals possible were used in the research.[16]

Evaluation of analgesic activity. The synthesized compounds **2** and **3** were evaluated for their *in vivo* analgesic efficacy by employing Swiss albino mice (*Mus musculus*), and the results were compared with that of the parent molecule piperine (**1**) and standard diclofenac sodium as well.

Peripheral analgesic activity. To investigate the peripheral analgesic efficacy, the acetic acid-induced writhing method was used.[17,18] Forty mice were used in the test, where five mice were randomly selected for each group. The negative control group received 0.9% NaCl, the positive control group received standard diclofenac sodium (25 mg/kg), while the compounds (**1**), (**2**), and (**3**) at 25 and 50 mg/kg doses were administered orally to the remaining groups.[19,20] After thirty min, each group was injected with 0.7% v/v acetic acid intraperitoneally to cause writhing in the experimental animals, which is an index of pain sensation. Five min later, the writhing counting for each mouse was started and recorded for the next fifteen min. By comparing the average writhing count of groups receiving the test samples and diclofenac sodium against that of the negative control, the percent inhibition of writhing was determined. Analgesia was measured by comparing the percentage of writhing inhibition of the test groups to the negative control group and the following formula was used to ascertain the % inhibition of writhing:

$$\% \text{ Inhibition} = \frac{(\text{Mean writhing of control group} - \text{Mean writhing of test group})}{\text{Mean writhing of control group}} \times 100$$

Central analgesic activity. Utilizing a hot water bath of 55°C, the tail immersion method was employed to examine the central analgesic property of compounds **1-3**.[21,22] Eight groups of mice were formed to carry out this experiment, each group consisting of randomly selected five mice. In this experiment, the positive control group obtained 2 mg/kg of morphine, while the negative control group received 0.9% NaCl. Rest of the groups received the test compounds at 25 and 50 mg/kg doses. At varied intervals (0, 30, 60 and 90 min), the tail flicking time was recorded as an indicator of the central analgesic activity. In comparison to the negative control, the percent (%) elongation of tail flicking time was calculated. The larger the central analgesic effect of the compound, the higher is its elongation percentage. The central analgesic activity of the test compounds was examined using the formula below:

$$\% \text{ Time elongation} = \left\{\frac{(At - Ac)}{Ac}\right\} \times 100$$

Wherein, At = Average time of tail flicking due to test compounds, Ac = Average time of tail flicking due to control.

Evaluation of anti-inflammatory activity. The anti-inflammatory property of the compounds **1-3** was investigated using carrageenan-induced hind paw edema technique in rats.[19,23] Twenty-five Wistar rats were randomly assigned into five groups, each group composing of five rats. Aceclofenac (100 mg/kg) and 0.9% NaCl were administered orally to the positive control and the negative control group, respectively.[24] The test compounds **1-3** were administered orally as suspension prepared in normal saline water as vehicle to the test groups at 100 mg/kg dose. At 0^{th} hour, volume of the right hind paw was determined (before injecting carrageenan) with a plethysmometer which served as a baseline reading. All groups of mice were injected with 0.1 ml of a 1% carrageenan solution in the right hind paw after 1 hour of oral treatment. After the administration of carrageenan, paw volumes were determined at 1, 2, 3, and 4 h. For the different time intervals, the average rise in the volume of the hind paw (injected) was determined.

The following formula was used to compute percent inhibition:

$$\% \text{ Inhibition} = \left\{\frac{(Vc - Vt)}{Vc}\right\} \times 100$$

Wherein, Vc = Average paw edema volume of rats in control group

Vt = Average paw edema volume of rats that received treatment

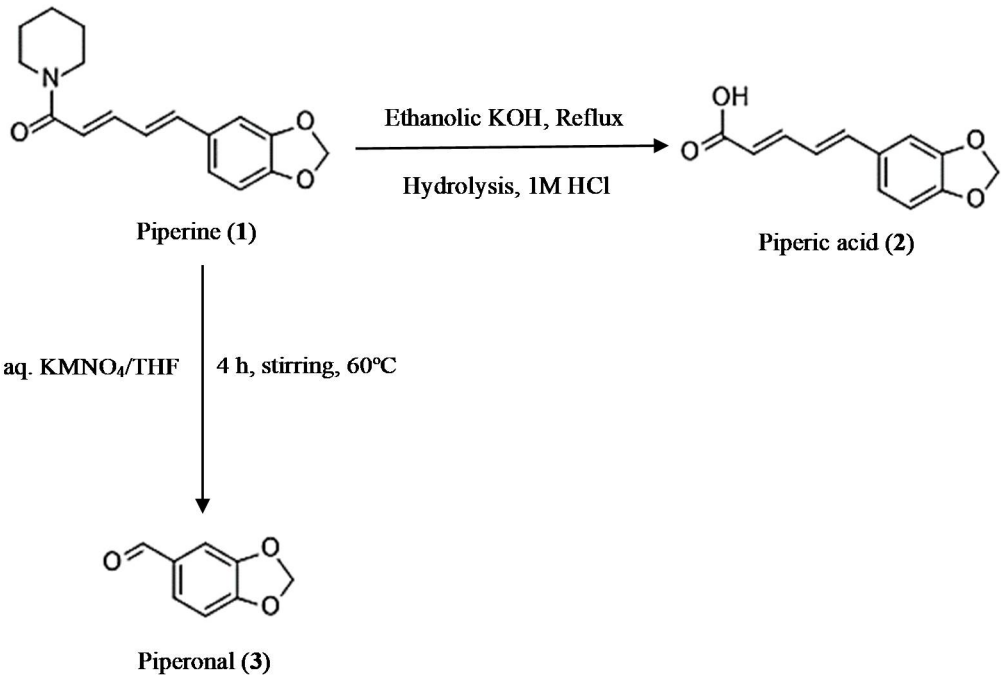
Statistical analysis. The mean ± standard error of the mean (SEM) was used to represent all the test outcomes, and the data were analyzed using GraphPad software, performing a one-way analysis of Variance (ANOVA) and then a Dunnett's test. Statistical values were deemed significant at $p < 0.05$.

RESULTS AND DISCUSSION

Isolation and purification of piperine. A modified extraction method by ethanol was used to isolate and purify piperine (**1**) from *P. nigrum* fruits and the yield of the pure crystalline compound was 3%. The purity of the compound was ascertained by TLC and the isolated piperine was analyzed by spectroscopic methods such as FTIR and ^{1}H NMR. The recorded ^{1}H NMR data resembled with that of the published data and therefore, further spectroscopic analysis was not performed.[15] The isolated piperine was used for the synthesis of few derivatives.

Synthesis of piperine derivatives. Piperine (**1**) is a natural lipophilic amide with an aromatic ring moiety, an aliphatic chain and a piperidine moiety connected to the side chain with amide linkage. These multifaceted features have made the molecule an attractive lead to synthesize a number of derivatives. A couple of derivatives have been synthesized with chemical modifications of the parent molecule (**1**) by employing simple, environmentally benign and cost-effective chemical reactions with high yield. The derivatives piperic acid (**2**) and piperonal (**3**) were produced by alkaline hydrolysis (Yield 76%) and oxidative cleavage (Yield 69%) of piperine, respectively (Scheme 1). FTIR and ^{1}H NMR spectroscopy were utilized for the characterization of the synthesized compounds. The spectral data were compared with the reported data of these compounds and found to be identical.[25,26] Interestingly, the absence of band at 1632.3 cm^{-1} in FTIR spectra which corresponded to the amide group of the piperine (**1**) confirms the complete conversion of piperine to piperic acid.

Analgesic activity. Two distinct approaches (peripheral and central) were utilized to ascertain the analgesic activity exerted by the isolated compound (**1**) and its synthetic analogs (**2**, **3**) . The acetic acid induced writhing method involves administering acetic acid intraperitoneally, which causes an immediate inflammatory response and the activation of nociceptors.[27] The tail immersion experimental technique is also used frequently to evaluate the anti-nociceptive potential of a compound. In this procedure, the transient receptor potential vanilloid type 1 and type 3 (TRPV1 and TRPV3), the heat-sensitive receptors, are enabled when mice tails are exposed to heat, enhancing the dissemination of pain.[28] In addition, this study is the first to document the analgesic effect of compound (**3**).



The synthesis of derivatives of piperine was conducted following the general synthetic scheme (Scheme 1).
Scheme 1. Synthesis of piperine derivatives from the isolated piperine from *P. nigrum* fruits.

Peripheral analgesic activity. Table 1 summarizes the peripheral analgesic potential of the synthetic compounds (**2**, **3**) in comparison to piperine (**1**) to lessen the pain caused by acetic acid. All the compounds exhibited substantial peripheral analgesic efficacy in a dose-dependent pattern. Compound **2** displayed remarkable analgesic action at a dose of 50 mg/kg, with percent inhibition of writhing value of 78% as compared with that of standard diclofenac sodium (25 mg/kg; 85%). This finding also suggests that piperic acid (**2**) can be a more effective analgesic agent than piperine (**1**) (writhing inhibition 74%), at a dose of 50 mg/kg. When given at 25 mg/kg dose, the compounds (**1**, **2**, and **3**) indicated satisfactory analgesic efficacy with inhibition values of 68%, 63%, and 62%, respectively. All the data were statistically significant ($p < 0.001$) and comparable to that of standard analgesic drug diclofenac sodium.

A delicate method for determining nociception in experimental animals is via the acetic acid induced abdominal writhing response. The response is predominantly mediated by the peritoneal macrophages as well as mast cells, that secrete cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin 1 (IL-1), along with interleukin 8 (IL-8) under the direction of prostaglandin pathways.[29] Diclofenac sodium employed in this trial as a standard is a nonsteroidal anti-inflammatory medication (NSAID) that inhibits cyclooxygenase (COX) enzyme, specifically COX-2, to prevent prostaglandin formation and exert antinociceptive activity.[30] As the antinociceptive efficacy of the piperine-derivatives was comparable to that of the standard, it is possible that they contain analgesic elements that can disrupt the prostaglandin pathways.[18,31] Furthermore, the structural similarity to piperine may enhance the outcomes of

antinociceptive screening of piperic acid and piperonal, which can further be exploited in future drug development studies.

Central analgesic activity. The tail immersion method was utilized to investigate the alterations in the susceptibility of the test animals caused by the central analgesic effect of the test compounds (**1-3**), and the results were presented in table 2. Following oral administration, compound **3** at 25 mg/kg dose, demonstrated superior analgesic effect as compared to compounds **1** and **2**, with percent tail flicking elongation times of 194%, 178%, and 178% at 30, 60, and 90 min, respectively. However, when the higher dose (50 mg/kg) was considered, compound **2** was found having the highest analgesic action in contrast to other compounds. At the dose of 50 mg/kg, the percentage tail-flick elongation time shown by compound **2** were 231%, 213%, and 206% at 30, 60, and 90 min, respectively, at $p < 0.001$.

To distinguish between central and peripheral analgesic activities, a reliable method is the tail immersion method. In this study, standard morphine was utilized, which primarily acts as a μ opioid receptor (MOR) agonist, yet it also has certain effects on the delta opioid receptor and kappa opioid receptor.[32] Since the findings acquired from the investigational compounds by employing the tail immersion method are almost similar to those of the standard morphine, it is conceivable that the synthetic derivatives **2** and **3** may provide an interfering agonistic function with the opioid receptors. Further research is required to ascertain the mechanism by which these compounds exert the analgesic effect, and additional structural alterations to piperine may result in more effective analgesic drugs.

Anti-inflammatory activity. The anti-inflammatory potential of the synthesized compounds was assessed by employing laboratory test animals, using a carrageenan-induced paw edema method. A 1% sub-plantar injection of carrageenan causes localized edema that develops over time and releases a variety of inflammatory mediators in two stages. Histamine, bradykinin, 5-hydroxytryptamine and platelet activating factor are released during the first phase, which can persist for as long as 1.5 hours following the administration of carrageenan, while prostaglandin and different cytokines predominate during the second phase.[33] The mean paw volume (ml) and percentage of paw edema inhibition by the test substances are depicted in table 3. It is evident from the data that, at the 1st, 2nd, 3rd and 4th h, compound **3** exhibited substantially greater anti-inflammatory effect with inhibition of paw edema, with values of 57%, 66%, 76%, and 81%, respectively in contrast to both compounds **1** (20%, 34%, 51%, 60%) and **2** (5%, 19%, 39%, 47%) when all compounds were compared against the standard aceclofenac (61%, 72%, 78%, 89%).

The current work is the first to disclose the *in vivo* anti-inflammatory property of piperonal (**3**). The NSAID aceclofenac, which has been used as the standard for the anti-inflammatory property evaluation, is a derivative of phenylacetic acid, exhibiting distinct analgesic and anti-inflammatory effects.[34] It has a strong inhibitory effect on cyclooxygenase (COX), a crucial enzyme in the production of prostaglandins and thromboxane that preferentially targets the COX-2 isoform.[35] However, gastrointestinal issues (dyspepsia, stomach discomfort, nausea and diarrhea), dizziness and elevated liver enzymes are the most frequent adverse events associated with aceclofenac found from clinical investigations and post-market monitoring experiences.[36] This study demonstrated that the isolated and synthesized compounds (**1**, **2**, **3**) exhibited various levels of anti-inflammatory effect, which may be because of their interaction with inflammatory mediators or interruption in any of the pathways that these mediators use to function, including antagonistic action on their receptors or disruption in their biosynthesis. Consequently, piperonal (**3**) may serve as a conceivable starting point for the design of prospective anti-inflammatory drugs with minimal side effects by bringing about advanced structural changes and subsequent pharmacological screenings.

Table 1. Assessment of peripheral analgesic activity of piperine (1), piperic acid (2) and piperonal (3) by acetic acid-induced writhing reaction in mice.

Sample ID	No. of writhing (Mean ± SEM)[a]	Writhing (%)	Inhibition (%)
CS	22.8 ± 0.604	100	-
SS	3.4 ± 0.430***	14.91	85.09
1 (d_1)	7.2 ± 1.562***	31.58	68.42
1 (d_2)	6.0 ± 1.304***	26.32	73.68
2 (d_1)	8.4 ± 1.806***	36.84	63.16
2 (d_2)	5.0 ± 1.304***	21.93	78.07
3 (d_1)	8.6 ± 0.872***	37.72	62.28
3 (d_2)	6.6 ± 0.872***	28.95	71.05

[a]Each value denotes Mean ± SEM, (n = 5); ***p < 0.001; **p < 0.01; *p < 0.05 compared with control (One-way ANOVA followed by Dunnett's test); CS = Control sample (0.9% NaCl solution); SS = Standard sample (Diclofenac sodium, 25 mg/kg b.w.); (d_1) = Lower dose (25 mg/kg b.w.); (d_2) = Higher dose (50 mg/kg b.w.)

Table 2. *In vivo* central analgesic property of piperine (1), piperic acid (2) and piperonal (3) in mice.

Sample ID	Tail flicking time (Mean ± SEM)[a]				% Elongation time			
	0 min.	30 min.	60 min.	90 min.	0 min.	30 min.	60 min.	90 min.
CS	0.86 ± 0.121	0.87 ± 0.120	0.86 ± 0.116	0.83 ± 0.101	-	-	-	-
SS_M	1.56 ± 0.124**	3.29 ± 0.155***	2.98 ± 0.131***	2.16 ± 0.112***	81.40	278.16	246.51	160.24
1 (d_1)	1.34 ± 0.119*	2.36 ± 0.106***	1.98 ± 0.134***	1.54 ± 0.108**	55.81	171.26	130.23	85.54
1 (d_2)	1.42 ± 0.171*	2.66 ± 0.139***	2.17 ± 0.175***	1.59 ± 0.140**	65.12	205.75	152.33	91.57
2 (d_1)	1.45 ± 0.073**	2.22 ± 0.117***	1.92 ± 0.115***	1.51 ± 0.0986**	68.60	155.17	123.26	81.93
2 (d_2)	2.32 ± 0.098***	2.88 ± 0.111***	2.69 ± 0.121***	2.54 ± 0.067***	169.77	231.03	212.79	206.02
3 (d_1)	2.28 ± 0.116***	2.56 ± 0.143***	2.39 ± 0.126***	2.31 ± 0.118***	165.12	194.25	177.91	178.31
3 (d_2)	2.30 ± 0.116***	2.45 ± 0.099***	2.40 ± 0.098***	2.31 ± 0.093***	167.44	181.61	179.07	178.31

[a]Each value is a representative of Mean ± SEM (n = 5), ***p < 0.001, **p < 0.01, *p < 0.05 compared with control (one-way ANOVA followed by Dunnett's test); CS = Control sample (0.9% NaCl solution); SS_M = Standard sample (Morphine, 2 mg/kg b.w.); (d_1) = Lower dose (25 mg/kg b.w.); (d_2) = Higher dose (50 mg/kg b.w.).

Table 3. *In vivo* anti-inflammatory activity of piperine (1), piperic acid (2) and piperonal (3) in rat model.

Sample ID	Paw volume (ml) (Mean ± SEM)[a]				% Paw edema inhibition			
	1^{st} hr	2^{nd} hr	3^{rd} hr	4^{th} hr	1^{st} hr	2^{nd} hr	3^{rd} hr	4^{th} hr
CS	0.79 ± 0.024	0.82 ± 0.023	0.84 ± 0.023	0.88 ± 0.026	--	--	--	--
SS_A	0.53 ± 0.017***	0.49 ± 0.015***	0.47 ± 0.011***	0.42 ± 0.013***	61.36	72.34	77.55	88.68
1	0.65 ± 0.032**	0.61 ± 0.042**	0.54 ± 0.033***	0.51 ± 0.032***	20.45	34.04	51.02	60.38
2	0.74 ± 0.026	0.70 ± 0.023**	0.62 ± 0.029***	0.60 ± 0.028***	4.55	19.15	38.78	47.17
3	0.39 ± 0.030***	0.36 ± 0.035***	0.32 ± 0.031***	0.30 ± 0.029***	56.82	65.96	75.51	81.13

[a]Each value indicates Mean ± SEM (n = 5), ***p < 0.001, **p < 0.01, *p < 0.05 compared against control (one-way ANOVA followed by Dunnett's test); CS = Control sample (0.9% NaCl solution), SS_A = Standard sample (Aceclofenac), dose = 100 mg/kg b.w.

CONCLUSION

In this study, piperine was extracted from black pepper with solvent in good amount employing solvent extraction method, and by means of alkaline hydrolysis and oxidative cleavage, two pharmacologically interesting derivatives were synthesized with satisfactory yields. Overall, the synthetic derivatives exhibited better pharmacological activities in dose dependent manner compared to the parent piperine, and the activities were comparable to the standard drugs used in these studies. Compound **2** was found to be the most active analgesic compound, and compound **3** was found to possess the highest anti-inflammatory activity. Hence, more research is needed to confirm these early findings and examine their mechanism of action and structure activity relationship in order to develop new analgesic and anti-inflammatory agents from these compounds.

REFERENCES

1. Butt, M. S., Pasha, I., Sultan, M. T., Randhawa, M. A., Saeed, F. and Ahmed, W. 2013. Black Ppepper and health claims: a comprehensive treatise. *Crit. Rev. Food Sci. Nutr.* **53**, 875-886.
2. Samykutty, A., Shetty, A. V., Dakshinamoorthy, G., Bartik, M. M., Johnson, G. L., Webb, B., Zheng, G., Chen, A., Kalyanasundaram, R. and Munirathinam, G. 2013. Piperine, a bioactive component of pepper spice exerts therapeutic effects on androgen dependent and androgen independent prostate cancer cells. *PLOS ONE* **8**, e65889.
3. Santiago, V. S., Alvero, R. G. and Villaseñor, I. M. 2014. *Aedes aegypti* larvicide from the ethanolic extract of *piper nigrum* black peppercorns. *Nat. Prod. Res.* **29**, 441-443.
4. Chonpathompikunlert, P., Wattanathorn, J. and Muchimapura, S. 2010. Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of alzheimer's disease. *Food Chem. Toxicol.* **48**, 798-802.
5. Tiwari, A., Mahadik, K. R. and Gabhe, S. Y. 2020. Piperine: a comprehensive review of methods of isolation, purification and biological properties. *Med. Drug Discov.* **7**, 100027.
6. Mittal, R. and Gupta, R. L. 2000. *In vitro* antioxidant activity of piperine. *Methods Find. Exp. Clin. Pharmacol.* **22**, 271-274.
7. Srinivasan, K., Patole, P.S., Kaul, C.L. and Ramarao, P. 2004. Reversal of glucose intolerance by pioglitazone in high fat diet-fed rats. *Methods Find. Exp. Clin. Pharmacol.* **26**, 327-333.
8. Meghwal, M. and Goswami, T. K. 2013. *Piper nigrum* and piperine: an update. *Phytother. Res.* **27**, 1121-1130.
9. Tripathi, A. K. 2017. Novel methodology for the isolation of pure piperine from plant source through sonication. *J. Chem. Edu. Res. Prac.* **1**, 00001.
10. Goswami, A. and Malviya, N. 2020. Reassessing the restorative prospectives of the king of spices black pepper. *J. Drug Deliv. Ther.* **10**, 312-321.
11. Singh, I. and Choudhary, A. 2015. Piperine and derivatives: trends in structure-activity relationships. *Curr. Top. Med. Chem.* **15**, 1722-1734.
12. Almekinders, L. C. 1999. Anti-inflammatory treatment of muscular injuries in sport. *Sports Med.* **28**, 383-388.
13. Bost, J., Maroon, A. and Maroon, J. 2010. Natural anti-inflammatory agents for pain relief. *Surg. Neurol. Int.* **1**, 80.
14. Ramesh, A., Sundarraj, P. and Balamani, Dr. J. 2018. Synthesis and biological evaluation of 5-(3,4-methylenedioxyphenyl)-2,4-pentadienoic acid derivatives as potent antimicrobial and cytotoxicity agents. *IOSR J. Appl. Chem.* **11**, 51-57.
15. Moraru, A. C. 2019. Insights of the antimicrobial activity of piperine extracted from *Piper nigrum* L. *Farmacia* **67**, 1099-1105.
16. Charan, J. and Kantharia, N. D. 2013. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* **4**, 303-306.
17. Koster, R., Anderson, M. and De Beer, E. J. 1959. Acetic acid for analgesic screening. *Fed. Proc.* **18**, 412-417.
18. Saha, P., Brishti, S. R. and Rahman, S. M. 2020. Synthesis and evaluation of disubstituted benzimidazole derivatives as potential analgesic and antidiarrheal agents. *Indian J. Pharm. Sci.* **82**, 39-46.
19. Spriha, S. E., Rahman, F. I. and Rahman, S. M. 2021. Synthesis, *in vivo* and *in silico* analgesic and anti-inflammatory studies of α-D-ribofuranose derivatives. *Saudi Pharm. J.* **29**, 981-991.
20. Faujdar, S., Sharma, S., Sati, B., Pathak, A. K. and Paliwal, S. K. 2016. Comparative analysis of analgesic and anti-inflammatory activity of bark and leaves of *Acacia ferruginea* DC. *Beni Suef Univ. J. Basic Appl. Sci.* **5**, 70-78.
21. Grotto, M. and Sulman, F. G. 1967. Modified receptacle method for animal analgesimetry. *Arch. Int. Pharmacodyn. Ther.* **165**, 152-159.
22. Ben-Bassat, J., Peretz, E. and Sulman, F.G. 1959. Analgesimetry and ranking of analgesic drugs by the receptacle method. *Arch. Int. Pharmacodyn. Ther.* **122**, 434-447.
23. Winter, C., Risley, E. and Nuss, G. 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Exp. Biol. Med.* **111**, 544-547.
24. Ramesh, B. and Sumana, T. 2010. Synthesis and anti-inflammatory activity of pyrazolines. *J. Chem.* **7**, 514-516.

25. Yasir, A., Jahangir, M., Ur-Rehman, A., Ishtiaq, S. and Shahid, M. 2017. Antimicrobial, hemolytic and thrombolytic activities of some new n-substituted-2-({5-[(1e,3e)f-4-(1,3-benzodioxol-5-YL)-1,3-butadienyl]-1,3,4-oxadiazol-2-yl}sulfanyl) propanamides. *Trop. J. Pharm. Res.* **16**, 1973.
26. Zazeri, G., Povinelli, A. P., Le Duff, C. S., Tang, B., Cornelio, M. L. and Jones, A. M. 2020. Synthesis and spectroscopic analysis of piperine- and piperlongumine-inspired natural product scaffolds and their molecular docking with il-1β and NF-KB proteins. *Molecules* **25**, 2841.
27. Dzoyem, J. P., McGaw, L. J., Kuete, V. and Bakowsky, U. 2017. In: *Medicinal Spices and Vegetables from Africa* (Kuete, V., Ed.), Academic Press, London, Chapter 9, pp. 239-270.
28. Leksiri, S., Hasriadi, H., Dasuni Wasana, P. W., Vajragupta, O., Rojsitthisak, P. and Towiwat, P. 2020. Co-administration of pregabalin and curcumin synergistically decreases pain-like behaviors in acute nociceptive pain murine models. *Molecules* **25**, 4172.
29. Ribeiro, R. A., Vale, M. L., Thomazzi, S. M., Paschoalato, A. B. P., Poole, S., Ferreira, S. H. and Cunha, F. Q. 2000. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *Eur. J. Pharmacol.* **387**, 111-118.
30. Cashman, J. N. 1996. The mechanisms of action of nsaids in analgesia. *Drugs* **52**, 13-23.
31. Karunakar, P., Krishnamurthy, V., Girija, C. R., Krishna, V., Vasundhara, D. E., Begum, N. S. and Syed, A. A. 2012. In silico docking analysis of piperine with cyclooxygenases. *J. Biochem. Tech.* **3**, 122-127.
32. Pathan, H. and Williams, J. 2012. Basic opioid pharmacology: an update. *Br. J. Pain* **6**, 11-16.
33. Mehrzadi, S., Khalili, H., Fatemi, I., Malayeri, A., Siahpoosh, A. and Goudarzi, M. 2020. Zingerone mitigates carrageenan-induced inflammation through antioxidant and anti-inflammatory activities. *Inflammation* **44**, 186-193.
34. Brogden, R. N. and Wiseman, L. R. 1996. Aceclofenac. *Drugs* **52**, 113-124.
35. Dooley, M., Spencer, C. M. and Dunn, C. J. 2001. Aceclofenac. *Drugs* **61**, 1351-1378.
36. Iolascon, G., Giménez, S. and Mogyorósi, D. 2021. A review of aceclofenac: analgesic and anti-inflammatory effects on musculoskeletal disorders. *J. Pain Res.* **14**, 3651-3663.