Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Phytol anti-inflammatory activity: Pre-clinical assessment and possible mechanism of action elucidation

Muhammad Torequl Islam[1,2], Seyed Abdulmajid Ayatollahi[3,4], S. M. Neamul Kabir Zihad[6,7], Nazifa Sifat[6], Md. Roich Khan[7], Arkajyoti Paul[7], Bahare Salehi[5*], Tawhida Islam[8], Mohammad S. Mubarak[9], Natália Martins[10,11], Javad Sharifi-Rad[3*]

[1] Department for Management of Science and Technology Development, Ton Duc Thang University, Ho Chi Minh City-700000, Vietnam
[2] Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City-700000, Vietnam
[3] Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Department of Pharmacognosy and Biotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5] Student Research Committee, School of Medicine, Bam University of Medical Sciences, Bam, Iran
[6] Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh
[7] Department of Pharmacy, Atish Dipankar University of Science and Technology, Uttara, Dhaka-1230, Bangladesh
[8] Department of Pharmacy, Life Science Faculty, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj-8100, Bangladesh
[9] Department of Chemistry, The University of Jordan, Amman 11942, Jordan
[10] Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, Porto 4200-319, Portugal
[11] Portugal Institute for Research and Innovation in Health (i3S), University of Porto, Porto 4200-135, Portugal

*Correspondence to: bahar.salehi007@gmail.com; mmubarak@ju.edu.jo; javad.sharifirad@gmail.com

Received April 10, 2020; **Accepted** May 14, 2020; **Published** June 25, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.4.31

Abstract: Phytol (PHY) is an acyclic natural diterpene alcohol and a chlorophyll constituent that exhibits several pharmacological effects, such as anticancer, antioxidant, and antimicrobial. Here, we aimed to assess the PHY anti-inflammatory effect *in vitro* and *in vivo*, and to deepen knowledge on the possible mechanism of action. For this purpose, egg albumin (*in vitro*) test was performed by using acetyl salicylic acid (ASA) as a standard nonsteroidal anti-inflammatory drugs (NSAID). For *in vivo* test, male Wistar albino rats were treated (intraperitoneally) with 100 mg/kg of PHY and/or standard NSAIDs ASA (100 mg/kg) and diclofenac sodium (Diclo-Na, 10 mg/kg) to evaluate the combined effect of PHY in formalin-induced paw edema model. Furthermore, an *in silico* (CADD) study was accomplished to assess the effect of PHY against cyclooxygenase (COX)-1 and 2 enzymes, nuclear factor kappa B (NF-κB), and interleukin-1β (IL-1β). Results revealed that PHY exhibits dose-dependent anti-inflammatory effect using the egg albumin method. PHY (100 mg/kg) co-treated with ASA and/or Diclo-Na reduced paw edema better than PHY alone or NSAIDs individual groups. Computational study showed that PHY efficiently interacts with COX-1 and 2, NF-κB, and IL-1β. In conclusion, PHY exhibits anti-inflammatory activity, possibly *via* COX-1 and 2, NF-κB, and IL-1β dependent pathways.

Key words: Phytol; anti-inflammatory effect; COX enzymes; NF-κB; IL-1β pathway.

Introduction

Phytol (PHY) is an acyclic natural diterpene alcohol and a constituent of chlorophyll abundantly found in nature (1). Although it is used mainly as a fragrance component, PHY possesses numerous important biological effects, drowning the attention of scientists for possible biomedical applications (2).

Research findings have shown that several inflammatory mediators, among them interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α), are involved in the nociceptive response to chemical stimulus in experimental animals (3,4). In this context, prostaglandins synthesis displays a key role in both inflammatory and febrile response to endogenous (e.g., cytokines) or exogenous (e.g., lipopolysaccharide) pyrogens (5). On the other hand, cyclooxygenase (COX) enzymes are involved in inflammation- and pain-related symptoms (6). Along this line, nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin (acetyl salicylic acid, ASA) and diclofenac (e.g., Diclofenac sodium, Diclo-Na) exert their effect by inhibiting the COX activity. Brain endothelium COX-2 induction exerts a crucial role in inflammation (7). COX-2 pathways transcription and induction are activated by cytokines and TNF, triggering the central mechanisms that act *via* the transcription factors such nuclear factor kappa B (NF-κB) and signal transducer and activator of transcription (STAT-3) (8,9). In male ICR and BALB/c mice, PHY inhibit allergic cytokine expression through NF-κB and activator protein (AP)-1 regulation (10). Moreover, Silva et al. (2014) suggested that PHY exhibits anti-inflammatory effect in carrageenan, compound 48/80-, histamine-, serotonin-, bradykinin- and PGE2-induced

paw edema models (11). Also, PHY inhibit neutrophil migration through reducing IL-1β and TNF-α levels and oxidative stress (11). Thus, in light of the wide range of PHY pharmacological profile, this study aimed to assess its anti-inflammatory potential both *in vitro* and *in vivo*. Additionally, the anti-inflammatory mechanism of action was investigated through combination of PHY with ASA or Diclo-Na and by an *in-silico* study. Results of this study may have some pharmacological implications as to the use of PHY as anti-inflammatory.

Materials and Methods

Drugs, chemicals and reagents

ASA and Diclo-Na were from Zenith Pharmaceuticals Ltd., Bangladesh. PHY and other analytical grade chemicals or reagents were acquired in Sigma Aldrich, USA.

Animals and diets

Adult male Wistar albino rats (120–150 g) were from the animal resource branch of Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong. Animals were housed at standard environmental conditions (temperature: 24±1 °C, humidity: 50±5%, and 12 h light/dark cycles), had free access to standard pellets (basal diet) and water *ad libitum*. Acclimatization phase was of 7 days before the beginning of the study. Then, animals were randomly divided into experimental (EG) and control (CG) groups, and food was withdrawn 12 h before the experiment's onset. Committee on Animal Research at the Federal University of Piauí (UFPI, #109/2014) approved all the procedures, which were done in accordance with the Brazilian (COBEA-Colégio Brasileiro de Experimentação Animal) and International Standards on experimental animals care and use (Directive 2010/63/EU of the European Parliament and of the Council).

Egg albumin test (in vitro)

This study was accomplished following the procedure of Ullah et al. (2014), with some modifications (12). Briefly, PHY (mixed with the vehicle 0.05% Tween 80 dissolved in 0.9% NaCl solution) was used at 12.5, 25, 50, 75 and 100 µg/mL. ASA (a standard) was dissolved in distilled water and employed at 100 µg/mL, while 0.05% Tween 80 (a vehicle) was dissolved in 0.9% NaCl solution. Reaction mixture (5 mL) was composed by egg albumin (0.2 mL), PBS (2.8 mL; pH 6.4), and test sample/controls (2 mL). Then, mixtures were incubated at 37±2 °C in a BOD incubator (Lab line Technologies) for 15 min and afterwards heated at 70 °C for 5 min. After cooling, absorbance was measured at 660 nm (LABOCON MODEL: LUVS-201, monochromatic beam) using the vehicle as a blank. The percentage of protein denaturation inhibition was determined using the following formula:

$$\% \text{ Inhibition} = [(A_{control} - A_{test}) \div A_{control}] \times 100$$

Formalin-induced paw edema test (in vivo)

The method of Chau (1989) was used to this experiment, with slight changes (13). Paw edema was induced through injection of 0.2 mL of 1% w/v formalin solution suspended in distilled water into the rat sub-plantar tissues of the left hind paw. For this purpose, 40 rats were divided into 8 groups with 5 animals each, as follows: G1 (formalin), G2 (formalin + Vehicle), G3 (ASA), G4 (Diclo-Na), G5 (PHY), G6 (PHY + ASA), G7 (PHY + Diclo-Na) and G8 (PHY + ASA + Diclo-Na). The last one was used for further confirmation of the co-treatment effects of PHY and standard NSAIDs. In all of these treatments (except formalin), animals were administered i.p. at 10 mL/kg (Table 2). Treatments were given 15 min before injecting the formalin solution. Thickness of the paw was measured in each rat by using a vernier caliper in mm.

Molecular docking study (in silico)

Four-dimensional crystal structures of COX-1 (PDB ID: 2OYE), COX -2 (PDB ID: 6COX), NF-κB (PDB ID: 5LDE), and IL-1β (PDB ID: 3O4O) were downloaded from the protein data bank; structures were prepared and refined by using Schrödinger-Maestro v10.1. Bond orders and charges were assigned, hydrogen moieties were added, selenomethionines were converted to methionines, and water molecules other than those present in the active site were removed. Optimization of amide groups on asparagines, imidazole ring and glutamines on histidines, protonation states on histidines, glutamic and aspartic acids was performed at neutral pH (7.0). Furthermore, prepared proteins were minimized to the RMSD value of 0.30 Å using the force field OPLS_2005. Docking grids of 17 Å × 17 Å × 17 Å were generated in glide around the active site residues (reference ligand active site) keeping the Van der Waals scaling factor 1.00 and charge cutoff 0.25 subjected to OPLS 2001 force field. The target compound, PHY, was retrieved from the Pubchem database (CID: 5280435) and the 3D structure was created by Schrödinger-Maestro v10.1 with OPLS 2005 force field, whereas PHY ionization states were generated at pH 7.0.

Flexible docking was achieved using the Glide of Schrödinger-Maestro v10.1 and applying penalties for non-*cis*/*trans* amide bonds and keeping all docking parameters as standard. No binding restrictions were given during the docking calculations. Ligand pose generated for the input molecule (PHY) and the PHY binding affinity to the target bio-macromolecules were calculated as docking glide score using Monte Carlo random search algorithm, performing post-docking minimization with the field OPLS 2005 force.

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) analysis

To evaluate whether PHY is biologically suitable to be considered as a drug, its pharmacokinetic parameters were analyzed. For this purpose, Swiss ADMET analysis (http://www.swissadme.ch/) was employed for estimating PHY ADMET.

Statistical analysis

Data were expressed as mean±SEM and analyzed using one-way analysis of variance (ANOVA), followed by Tukey post hoc multiple comparison test. GraphPad Prism software (v. 6.0, San Diego, California, USA) was used for statistical analysis, with $p<0.05$ considered statistically significant.

Table 1. Inhibition of egg albumin by PHY and controls.

Treatments		%IPD	EC_{50} [CI; R^2]
Vehicle		1.08±0.08	-
ASA (100 µg/mL)		78.08±1.08*	-
	12.5	18.12±0.08*	
	25	23.12±0.10*	
PHY (µg/ml)	50	32.63±0.19*a	88.92 ± 0.79 [40.35–117.70; 0.77]
	75	38.46±0.58*ab	
	100	62.12±0.78*abcd	

p <0.05 when compared to the *Control, [a]PHY12.5, [b]PHY25, [c]PHY50 and [d]PHY75. ASA: Acetyl salicylic acid; CI: Confidence interval; EC_{50}: Median effective concentration; IPD: Inhibition of protein denaturation; PHY: Phytol; R^2: Co-efficient of determination.

Table 2. Day wise effect of PHY and/or standard NSAIDs compared to formalin control group in formalin-induced paw edema model using vernier calipers.

Treatment groups	Dose (i.p.) at 10 ml/kg	Change in paw thickness in mm at different days		
		1st day	2nd day	3rd day
Formalin (NC)	0.2 ml of 2%w/v	4.95 ± 0.18	4.10 ± 0.21	3.10 ± 0.009
NC + Vehicle	-	4.80 ± 0.08	4.05 ± 0.18	3.08 ± 0.58
ASA	100 mg/kg	3.70 ± 0.95*#c	2.58 ± 0.08*#c	1.79 ± 0.20*#
Diclo-Na	10 mg/kg	3.50 ± 0.22*#ac	2.43 ± 0.15*#ac	1.58 ± 0.19*#ac
PHY	100 mg/kg	3.96 ± 0.11*#	2.78 ± 0.19*#	1.92 ± 0.05*#
PHY + ASA	100 + 100 mg/kg	3.09 ± 0.10*#abc	2.01 ± 0.08*#abc	1.53 ± 0.04*#ac
PHY + Diclo-Na	100 + 10 mg/kg	3.02 ± 0.04*#abc	1.90 ± 0.15*#abc	1.40 ± 0.03*#abcd
PHY + ASA + Diclo-Na	100 + 100 + 10 mg/kg	2.96 ± 0.03*#abc	1.07 ± 0.18*#abcde	0.9 ± 0.12*#abcde

p <0.05 when compared to the corresponding day of *Negative control (Formalin), #NC + Vehicle, [a]ASA, [b]Diclo-Na, [c]PHY and [d]PHY+ASA. [e]PHY+Diclo-Na. ASA: Acetyl salicylic acid; Diclo-Na: Diclofenac sodium; PHY: Phytol.

Results

Results revealed that PHY, at all doses tested, markedly inhibits heat-induced protein denaturation in egg albumin method. ASA, at 100 µg/mL, exhibited the highest protein denaturation inhibition (78.08±1.08), whereas the same dose of PHY led to 62.12±0.78 inhibition. On the other hand, the vehicle showed negligible protein denaturation inhibition as shown in Table 1.

Results from Table 2 suggest that PHY and both NSAIDs significantly ($p<0.05$) reduce paw edema in a time-dependent manner comparing with control and vehicle groups. Diclo-Na was found to reduce more paw volume than ASA and PHY groups. Interestingly, PHY when co-administered with ASA or Diclo-Na was found to be more potent in reducing paw edema than PHY, ASA, and Diclo-Na groups. However, a significant and best reduction of paw edema was observed in PHY + ASA and in PHY + Diclo-Na groups. The highest reduction of paw volume was 0.9 ± 0.12 mm on the 3rd day in PHY + ASA + Diclo-Na group.

Results from *in silico* study suggests that PHY strongly interacts with COX-2 (binding affinity: –3.778, followed by NF-κB, COX-1, and IL-1β with binding affinities of -1.979, -1.583, and -0.379, respectively. PHY was docked in the reference binding pockets of these proteins (Figure 1) and the interacting bond types are shown in Table 3. In addition, results suggest that, PHY interacts with 10 amino acids within the reference binding pocket when docked with COX-2, whereas it interacts with 8, 5, and 5 amino acid residues, respectively, when docked with NF-κB, COX-1, and IL-1β. These interactions involved 4 types of attractive forces, including alkyl bond, pi-alkyl bond, carbon-carbon bond, and conventional hydrogen bond.

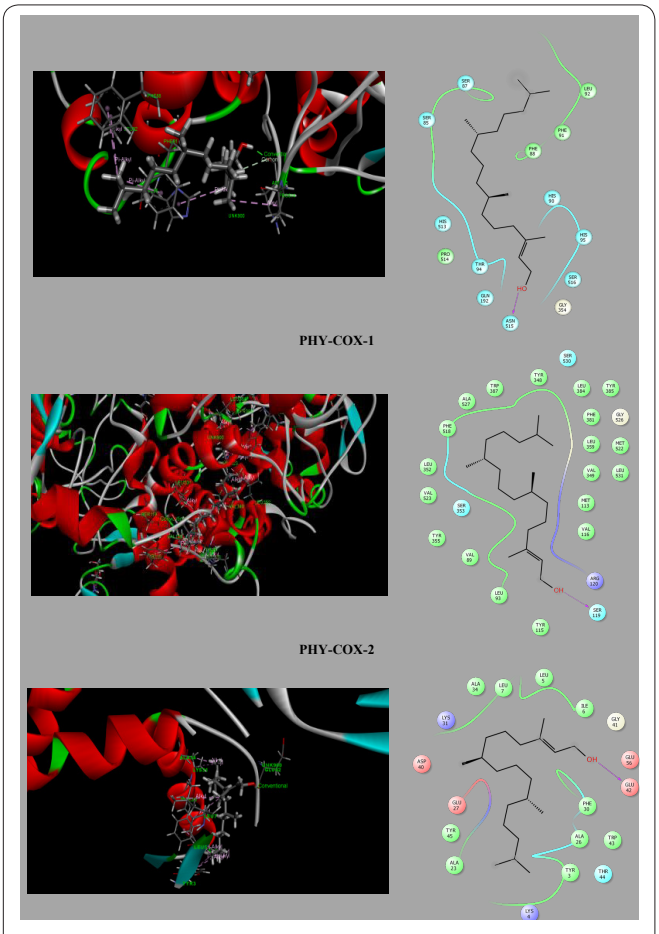

Figure 1. Molecular docking of phytol (PHY) with COX-1, COX-2, NF-κB, and IL-1β.

Furthermore, we examined the drug-like property

Table 3. Results of molecular docking studies.

Target protein	Binding affinity (Kcal/mol)	Interacting amino acids (type of interaction) *
COX-1	-1.583	Phe91 (π-Alk), Leu92 (Alk), His95 (π-Alk), Pro514 (Alk), Asn515 (C-C, Con-H)
COX-2	-3.778	Val89 (Alk), Tyr115(Alk, π-Alk), Val116 (Alk), Ser119 (Con-H), Val349 (Alk), Leu352 (Alk), Leu359 (Alk), Leu384 (Alk), Trp387(π-Alk), Val523 (Alk)
NF-κB	-1.979	Tyr3 (π-Alk), Leu5 (Alk), Leu7 (Alk), Ala23 (Alk), Phe30 (Alk), Lys31 (Alk), Ala34 (Alk), Glu42 (π-Alk, Con-H)
IL-1β	-0.379	Ile49 (Alk), Tyr51 (π-Alk), Leu59 (π-Alk), Asn64 (Con-H), Phe65 (Con-H)

*Alk: Alkyl bond; π-Alk: Pi-alkyl bond; C-C: Carbon-carbon bond; Con-H: Conventional hydrogen bond.

Table 4. ADME/T properties of PHY.

Compound	Molecular weight[a]	H-bond donor[b]	H-bond acceptor[c]	Log P[d]	Molar refractivity[e]
Phytol	296.53 g/mol	1	1	6.23	98.94

ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; [a]Molecular weight (acceptable range <500 g/mol); [b]H-bond donor (acceptable range <5); [c]H-bond acceptor (acceptable range <10); [d]High lipophilicity (expressed as Log P, acceptable range <5); [e]Molar refractivity should be between 40 and 130.

of PHY by evaluation of its ADMET; results are given in Table 4. It was found that PHY can be considered a drug-like molecule upon analyzing its molecular weight, capacity of hydrogen bond donating or accepting, lipophilicity, and molar refractivity according to Lipinski's rule of 5.

Discussion

Natural products, whether in the form of pure compounds or standardized extracts of various sources, have gained much attention in the field of drugs discovery and development (14). In this context, diterpenes have been coming into the spotlight given their multiple bioactivities (15). PHY is a diterpenoid known for its fragrance indication and diverse pharmacological effects (1).

On the other hand, oxidative stress leads to inflammation, a key triggering factor of some known diseases, such as atrial fibrillation in obesity and diabetes (16), and in neurological diseases and disorders (17, 18). Currently available NSAIDs have many side effects, including combinational risks, cardiovascular diseases, erectile dysfunction, gastrointestinal complications, inflammatory bowel disease, renal diseases, photosensitivity, pregnancy disorders, allergic or allergic-like hypersensitivity reactions, anastomosis raised liver enzymes, headache, dizziness, hyperkalemia, confusion, complicated breathing, and aseptic meningitis, among others. In addition, diterpenes, such as carnosol (19), paclitaxel (20), and andographolide and its derivatives (21) have shown anti-inflammatory activity in various test systems. Generally, diterpenes are cytoprotective in nature and possess diverse pharmacological effects (15).

On the other side, oxidative stress leading to inflammation can damage cell macromolecules, such as carbohydrates, proteins, lipids, and genetic materials (e.g., DNA, RNA) (22). Results obtained from this study indicated that PHY, at all doses (*in vitro*), inhibits protein denaturation, which might be related to its antioxidant (22-24) and anti-inflammatory[11] activities. Furthermore, the *in vivo* data suggested that PHY alone or co-administered with NSAIDs exhibit anti-inflammatory effects in rats. In addition, research findings showed that PHY can led to a substantial reduction of IFN-γ, IL-4, and IL-10 production in mouse splenocytes stimulated by T-cell mitogens (25). PHY is also able to inhibit IL-17A production, a promoter of neuro-inflammation and cognitive dysfunction in animals through NF-κB signaling pathway activation (26). In this respect, a recent study by Goncalves and colleagues found that a betulin derivative inhibits IFN-γ and modulates COX-2 expression (27). In a similar fashion, the *Ephedra gerardiana* hidroethanolic extract and fractions down-regulated PGE2, COX-2, IL-1β, IL-6, TNF-α, and NF-κB, and upregulated IL-4 and IL-10 expression in Sprague Dawley rats (28). Thus, the immunomodulatory (10) and anti-inflammatory (11) effects of PHY in experimental animals may be interrelated. In a study, Costa et al. (2014) showed that PHY exerts anxiolytic-like effects in mice possibly through GABAergic transmission which is similar to the effect of diazepam (29). Along this line, diazepam was found to enhance the neuroprotective activity of NS-398, a COX-2 inhibitor in male Sprague-Dawley rats (30). Therefore, the PHY- mediated neuroprotective effects (29, 31-33) may be linked with each other. In this study, PHY co-treated with ASA and Diclo-Na exhibited more potent anti-inflammatory effect than PHY and NSAIDs individual groups, where both of these NSAIDs act through COX enzymes.

The *in silico* study indicated that PHY strongly interacts with COX-2, the enzyme responsible for inflammatory prostaglandins generation (9). Additionally, PHY showed significant interaction with NF-κB, a regulatory protein that mediates COX-2, TNF-α, and IL-1β expression (34). Furthermore, our results reveal a noticeable interaction of PHY with COX-1 and IL-1β. These results agree with that of Silva et al. (2014) that proposed IL-1β and TNF-α levels reduction, triggered by PHY, as a potential mechanism of its anti-inflammatory action (11). Possible anti-inflammatory mechanism of PHY is depicted in Figure 2.

Evaluation of the pharmacokinetic parameters (ADMET) is an important factor in drug discovery, since it reduces the likelihood of failures in clinical phases (35), and the computer-based approach is a reliable and convenient strategy to do so (36). In our study, PHY was found to be a potential drug-like molecule for oral administration due to its lower molecular weight and lower hydrogen bonding capacity. Low molecular weight and low hydrogen bonding capacity contribute to better per-

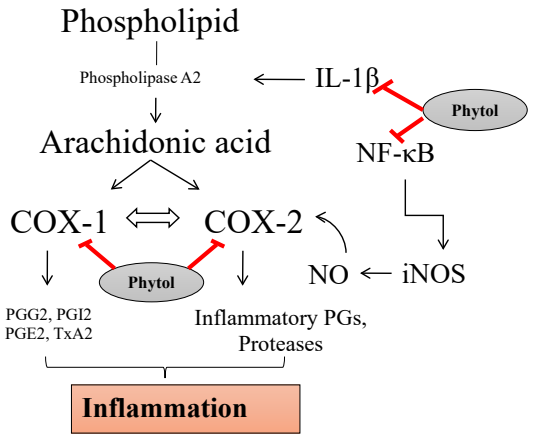

Figure 2. Phytol-mediated possible anti-inflammatory pathways. [PHY can inhibit COX-1 and COX-2 *via* suppressing interleukin-1beta (IL-1β) and nuclear factor kappa B (NF-κB). Moreover, PHY can also inhibit COX-1 and COX-2 which are responsible for inhibiting inflammatory mediators such as PGG2, PGI2, PGE2, TxA2, inflammatory PGs, and proteases].

meation, or absorption of orally delivered drug, thus increasing bioavailability (37, 38). However, lipophilicity (log P) value was found to be a bit over the optimum level, but it can be overcome by designing analogues. Hence, this investigation suggests that PHY might be a suitable candidate as an anti-inflammatory drug.

From this study, PHY exerts anti-inflammatory effect in heat-induced egg protein denaturation method and formalin-induced paw edema animals. PHY co-administered with ASA and/or Diclo-Na significantly reduced paw edema in experimental animals. Additionally, *in silico* study reveals that PHY has prominent interactions with COX-1 and -2, NF-κB and IL-1β. Also, this study suggests that PHY may exert its anti-inflammatory effect through COX-1 and -2, NF-κB, and IL-1β-dependent pathways.

Conflict of interest

The authors have no conflict of interests to declare.

References

1. Islam, M.T., Alencar, M.V.O.B., Machado, K.C., Machado, K.C., Melo-Cavalcante, A.A.C., et al. (2015) Phytol in a pharmamedico-stance. Chem.-Biol. Interact. 240, 60–73.
2. Islam, M.T., Ali, E.S., Uddin, S.J., Shaw, S., Islam, M.A., et al. (2018) Phytol: A review of biomedical activities. Food Chem. Toxicol. 121, 82–94.
3. Santos, A.R., Vedana, E.M., and De Freitas, G.A. (1998) Antinociceptive effect of meloxicam in neurogenic and inflammatory nociceptive models in mice. Inflam. Res. 47, 302–307.
4. Ribeiro, R.A., Vale, M.L., Thomazzi, S.M., Paschoalato, A.B.P., Poole, S., et al. (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.
5. Zhu, Z.Z., Ma, K.J., Ran, X., Zhang, H., Zheng, C.J., et al. (2011) Analgesic, anti-inflammatory and antipyretic activities of the petroleum ether fraction from the ethanol extract of *Desmodium podocarpum*. J. Ethnopharmacol. 133, 1126–1131.
6. Litalien, C., and Beaulieu, P. (2011) Chapter 117 – Molecular Mechanisms of Drug Actions: From Receptors to Effectors. In Fuhrman BP, Zimmerman JJ. Pediatric Critical Care (4th ed.). Philadelphia, PA: Elsevier Saunders. pp. 1553–1568.
7. Vidensky, S., Zhang, Y., Hand, T., Goellner, J., Shaffer, A., et al. (2003) Neuronal overexpression of COX-2 results in dominant production of PGE2 and altered fever response. Neuromol. Med. 3, 15–28.
8. Inoue, W., Somay, G., Poole, S., and Luheshi, G.N. (2008) Immune-to-brain signaling and central prostaglandin E2 synthesis in fasted rats with altered lipopolysaccharide-induced fever. Am. J. Physiol. Regul. Integrat. Comprehen. Physiol. 295, R133–143.
9. Zihad, S.M.N.K., Bhowmick, N., Uddin, S.J., Sifat, N., Rahman, M.S., et al. (2011) Analgesic activity, chemical profiling and computational study on *Chrysopogon aciculatus*. Front. Pharmacol. 9, 1164.
10. Ryu, K.-R., Choi, J.-Y., Chung, S., and Kim, D.-H. (2011) Anti-scratching behavioral effect of the essential oil and phytol isolated from artemisia princeps pamp. In mice. Planta Med. 77, 22–26.
11. Silva, R.O., Sousa, F.B.M., Damasceno, S.R.B., Carvalho, N.S., Silva, V.G., et al. (2014) Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fund. Clin. Pharmacol. 28, 455–464.
12. Ullah, H.M.A., Zaman, S., Juhara, F., Akter, L., Tareq, S.M.E.H., and Bhattacharjee, R. (2014) Evaluation of antinociceptive, *in-vivo* & *in-vitro* anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria* rhizome. BMC Complem. Alt. Med. 14, 346.
13. Chau, T.T. (1989) Analgesic testing in animal models. In: Alan R, editor. Pharmacological methods in the control of inflammation. New York: Liss Inc, U.K.
14. Eliaser, E.M., Ho, J.H., Hashim, N.M., Rukayadi, Y., Ee, G.C.L., and Razis, A.F.A. (2018) Phytochemical Constituents and Biological Activities of Melicope lunu-ankenda. Molecules. doi: 10.3390/molecules23102708
15. Islam, M.T., da Mata, A.M., de Aguiar, R.P., Paz, M.F., de Alencar, M.V., et al. (2016) Therapeutic Potential of Essential Oils Focusing on Diterpenes. Phytother. Res. 30, 1420–1444.
16. Karam, B.S., Chavez-Moreno, A., Koh, W., Akar, J.G., and Akar, F.G. (2017) Oxidative stress and inflammation as central mediators of atrial fibrillation in obesity and diabetes. Cardiovasc. Diabetol. 16, 120.
17. Muriach, M., Flores-Bellver, M., Romero, F.J., and Barcia, J.M. (2014) Diabetes and the brain: oxidative stress, inflammation, and autophagy. Oxid. Med. Cell Longev. 2014, 102158.
18. Dandekar, A., Mendez, R., and Zhang, K. (2015) Cross talk between ER stress, oxidative stress, and inflammation in health and disease. Meth. Mol. Biol. 1292, 205-214.
19. Zheng, Y., Zhang, Y., Zheng, Y., and Zhang, N. (2018) Carnosol protects against renal ischemia-reperfusion injury in rats. Exp. Anim. 67, 545-553.
20. Li, H., Wang, X., and Xu, A. (2018) Effect of Paclitaxel+Hirudin on the TLR4-MyD88 Signaling Pathway During Inflammatory Activation of Human Coronary Artery Smooth Muscle Cells and Mechanistic Analysis. Cell Physiol. Biochem. 50, 1301-1317.
21. Weng, Z., Chi, Y., Xie, J., Liu, X., Hu, J., et al. (2018) Anti-Inflammatory Activity of Dehydroandrographolide by TLR4/NF-κB Signaling Pathway Inhibition in Bile Duct-Ligated Mice. Cell Physiol. Biochem. 49, 1083-1096.
22. Islam, M.T., Streck, L., Paz, M.F.C.J., Sousa, J.M.C., Alencar, M.V.O.B., et al. (2016) Preparation of phytol-loaded nanoemulsion and screening for antioxidant capacity. Int. Arch. Med. 9, 1-15.
23. Santos, C.Cd.M.P., Salvadori, M.S., Mota, V.G., Costa, L.M., de Almeida, A.A.C., et al. (2013) Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Neurosci. J. 2013, 9.
24. Pejin, B., Savic, A., Sokovic, M., Glamoclija, J., Ciric, A., et al. (2014) Further *in vitro* evaluation of antiradical and antimicrobial activities of phytol. Nat. Prod. Res. 28, 372-376.

25. Nakanishi, T., Anraku, M., Suzuki, R., Kono, T., Erickson, L., and Kawahara, S. (2016) Novel immunomodulatory effects of phytanic acid and its related substances in mice. J. Funct. Foods 21, 283-289.
26. Yang, Z.Y., and Yuan, C.X. (2018) IL-17A promotes the neuroinflammation and cognitive function in sevoflurane anesthetized aged rats via activation of NF-κB signaling pathway. BMC Anesthesiol. 18, 147.
27. Gonçalves, S.M.C., Silva, G.N., Pitta, I.D.R., Rêgo, M.J.B.M., Gnoato, S.C.B., and Pitta, M.G.D.R. (2018) Novel betulin derivatives inhibit IFN-γ and modulates COX-2 expression. Nat. Prod. Res. 22, 1-10.
28. Uttra, A.M., Alamgeer, Shahzad, M., Shabbir, A., and Jahan, S. (2018) *Ephedra gerardiana* aqueous ethanolic extract and fractions attenuate Freund Complete Adjuvant induced arthritis in Sprague Dawley rats by downregulating PGE2, COX2, IL-1β, IL-6, TNF-α, NF-kB and upregulating IL-4 and IL-10. J. Ethnopharmacol. 224, 482-496.
29. Costa, J.P., de Oliveira, G.A.L., de Almeida, A.A.C., Islam, M.T., de Sousa, D.P., and de Freitas, R.M. (2014) Anxiolytic-like effects of phytol: possible involvement of GABAergic transmission. Brain Res. 1547, 34-42.
30. Trandafir, C.C., Pouliot, W.A., Dudek, F.E., and Ekstrand, J.J. (2015) Co-administration of subtherapeutic diazepam enhances neuroprotective effect of COX-2 inhibitor, NS-398, after lithium pilocarpine-induced status epilepticus. Neuroscience 284, 601-610.
31. Bang, M., Choi, S., Jang, T., Kim, S., Kwon, O., and Kang, T. (2002) Phytol, SSADH inhibitory diterpenoid of *Lactuca sativa*. Arch. Pharm. Res. (Seoul) 25, 643-646.
32. Costa, J.P., Ferreira, P.B., De Sousa, D.P., Jordan, J., and Freitas, R.M. (2012) Anticonvulsant effect of phytol in a pilocarpine model in mice. Neurosci. Lett. 523, 115-118.
33. Costa, J.P., Oliveira, J.S., Junior, L.M.R., and de Freitas, R.M. (2014) Phytol a natural diterpenoid with pharmacological applications on central nervous system: a review. Recent Pat. Biotechnol. 8, 194-205.
34. Tak, P.P., and Firestein, G.S. (2001) NF-κB: a key role in inflammatory diseases. J. Clin. Invest. 107, 7-11.
35. Hay, M., Thomas, D.W., Craighead, J.L., Economides, C., and Rosenthal, J. (2014) Clinical development success rates for investigational drugs. Nat. Biotechnol. 32, 40-51.
36. Dahlin, J.L., Inglese, J., and Walters, M.A. (2015) Mitigating risk in academic preclinical drug discovery. Nat. Rev. Drug Discov. 14, 279-94.
37. Duffy, F.J., Devocelle, M., and Shields, D.C. (2015) Computational approaches to developing short cyclic peptide modulators of protein-protein interactions. Meth. Mol. Biol. 1268, 241–271.
38. Lipinski, C.A., Lombardo, F., Dominy, B.W., and Feeney, P.J. (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 23, 3–25.