



Anti-inflammatory Properties of the Crude Extract of *Phyllostachys heterocykla* in Two Different Models of Acute Inflammation in Experimental Rats

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Abstract

The crude methanolic extract of *Phyllostachys heterocykla* was examined for the anti-inflammatory activity in rats using two different models of paw inflammation. Induction of edema was performed using 1% carrageenan in the first model and 2.5% formalin in the second model. The extract powder was dissolved in tween 80 and given orally for six consecutive days. Treatment with two doses (250 mg/kg and 500 mg/kg) of the extract of *Phyllostachys heterocykla* caused a dose-dependent reduction of paw thickness in both models. The anti-inflammatory effect of the crude extract of *Phyllostachys heterocykla* was also evaluated by determination of the serum levels of four inflammatory markers, where the serum levels of NF- κ B, TNF- α , IL-1 β and IL-6 were markedly increased in both models compared to the normal rats, with greater numbers recorded in the carrageenan-induced group. Treatment with both doses of the extract of *Phyllostachys heterocykla* led to a significant reeducation in all the detected markers in both the carrageenan and the formalin induced models, especially in case of the higher dose (500 mg/kg) that showed almost no significant different results compared to the normal group. In conclusion, the current preliminary study reports strong anti-inflammatory properties of *Phyllostachys heterocykla* extract.

Keywords: *Phyllostachys heterocykla*; rat paw edema; inflammatory markers; anti-inflammatory.

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1. Introduction

Bamboos are a diverse group of evergreen perennial flowering plants. They are of notable economic and cultural importance in Asia, being used as a source of building materials, a food, and a versatile raw product.

Previous studies on the medicinal activities of bamboo leaves proved their antibacterial properties (Tanaka et al., 2013). In folk medicine, they are used for their antipyretic and anti-inflammatory activities (Nirmala et al., 2011). Their usefulness in treatment of different cardiovascular disease was also reported (Park et al., 2007).

Additionally, they were proved to possess antioxidant activity (Park & Jhon, 2010; Zhang et al., 2008). Phenolic compounds (Kweon et al., 2001; Sarita et al., 2008), lignans (Suga et al., 2003) and volatile compounds (Takahashi et al., 2010) were mentioned as the major chemical constituents of bamboo species. We previously investigated the non-polar extract of *Phyllostachys heterocycla*. This study resulted in isolation and structure elucidation of seven compounds (Abdelhameed et al., 2020). In the present work, the crude methanolic extract of *Phyllostachys heterocycla* was examined for the anti-inflammatory activity in rats using two different models of paw inflammation.

2. Materials and Methods

2.1. Plant Material:

Phyllostachys heterocycla was collected from Isahaya, Nagasaki, Japan on October 2011. The plant was identified by Koji Yamada, Garden for Medicinal Plants, School of Pharmacy, Nagasaki University, Japan. An amount of 2 Kg was repeatedly extracted three times with methanol (5 L) at room temperature, and the combined extracts were concentrated in vacuum using a rotary evaporator to give a residue of 20 g which was kept in the refrigerator till used.

2.2. Experimental animals:

A total of fifty six (56) male albino rats (190 – 210 g) were purchased from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). They were left to acclimatize for ten days at $25 \pm 3^\circ\text{C}$ with a normal dark/light a cycle and free access to standard diet and tap water. The animals were kept and used following the guidelines of the National Research Council (2011). The study protocol was ethically approved by the Committee of Faculty of Pharmacy, Suez Canal University (201711MA₃).

2.3. Induction of inflammation:

Two different models of paw inflammation were induced. In the first model; 100 μL of 1% carrageenan dissolved in phosphate buffer saline (PBS) (Sigma-Aldrich, Germany) was injected subcutaneously into the planter region of the right hind limb of the rats. In the second model; rats' hind limbs were injected similarly with 100 μL of 2.5% formalin dissolved in PBS (Sigma-Aldrich, Germany). After 24 hours of injection, the induced

inflammation was assessed by measurement of the paw thickness using an electric caliper.

2.4. Study Design:

The rats were divided randomly into seven groups, each containing eight rats, as follows:

Group 1: Normal group; with no induced inflammation. The rats were injected with 100 μL of the PBS vehicle at the right hind limb.

Group 2: Carrageenan control group; received tween 80 as vehicle.

Group 3: Carrageenan + 250 mg/kg of *Phyllostachys heterocycla* extract.

Group 4: Carrageenan + 500 mg/kg of *Phyllostachys heterocycla* extract.

Group 5: Formalin control group; received tween 80 as vehicle.

Group 6: Formalin + 250 mg/kg of *Phyllostachys heterocycla* extract.

Group 7: Formalin + 500 mg/kg of *Phyllostachys heterocycla* extract.

The extract powder was dissolved in tween 80 and given orally. Treatment was started the day after induction of inflammation and continued for six consecutive days. 12 hours after the last dose, the paw thickness of each rat was re-measured and % reduction of inflammation was calculated in all rats by the following equation:

$$\% \text{ reduction of inflammation} = \frac{[(\text{paw thickness before treatment} - \text{paw thickness after treatment}) / \text{paw thickness before treatment}] * 100}{100}$$

2.5. Collection of samples:

After treatment for 6 consecutive days, rats were fasted over-night, and blood samples were collected from the orbital sinus of thiopental sodium anesthetized rats before scarification. Blood was centrifuged at 3000 rpm for 15 min to separate serum. Serum samples were kept at -20°C .

2.6. Biochemical measurements:

Enzyme linked immunosorbent assay (ELISA) kits were used to determine the serum levels of the inflammatory markers: nuclear factor kappa B (NF- κB) [Cat # MBS453975], tumor necrosis factor-

alpha (TNF- α) [Cat # MBS2507393], interleukin-1 beta (IL-1 β) [Cat # MBS825017], and interleukin-6 (IL-6) [Cat # MBS175908] (MyBioSource, USA).

2.7. Statistical analysis:

Data are represented as mean \pm SD. The results were compared by one-way analysis of variance (ANOVA) test followed by Bonferroni's post-hoc test for multiple comparisons. Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS), version 21.0 (IBM, USA). A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion:

In both models of inflammation, a pronounced paw swelling was observed, that was detected by measurement of paw thickness after administration of 1% carrageenan or 2.5% formalin. Treatment with two doses of the extract of *Phyllostachys heterocycla* caused a dose-dependent reduction of inflammation in both models as represented in **Figure 1**. The low dose (250 mg/kg) decreased the inflammation in both the carrageenan-induced and formalin-induced models by 18% and 26%, respectively, while the high dose (500 mg/kg) caused a more pronounced decrease of paw thickness in both the carrageenan model by 29% and the formalin model by 34%. It is clear that the anti-inflammatory effect was more obvious against formalin-induced inflammation.

The anti-inflammatory effect of the crude extract of *Phyllostachys heterocycla* was also evaluated by determination of the serum levels of four inflammatory markers, where the serum levels of NF- κ B, TNF- α , IL-1 β and IL-6 were markedly increased in both models compared to the normal rats, with greater numbers recorded in the carrageenan-induced group. The levels detected in carrageenan and formalin induced inflammation revealed an increase of the NF- κ B levels by 4.4 and 3.4 fold, respectively, where TNF- α levels increased by 3.9 and 3.3 fold, respectively. Levels of IL-1 β increased in the carrageenan administered group by 5.1 fold and in the formalin administered group by 4.1 fold, and levels of IL-6 increased by 4.2 and 3.8 fold in both groups, respectively. The increase in all the inflammatory markers was significantly different compared to the normal rats.

Figure 2 shows the effect of treatment with the extract of *Phyllostachys heterocycle* on the

inflammatory markers in the carrageenan administered group. The levels of all the inflammatory markers decreased significantly on treatment by both doses (250 mg/kg and 500 mg/kg). It was noticed that treatment by the higher dose (500 mg/kg) decreased the serum levels of the four markers significantly compared to the levels detected in the group treated with the lower dose (250 mg/kg). Interestingly, the levels of NF- κ B, TNF- α and IL-6 recorded in the group treated by 500 mg/kg of the *Phyllostachys heterocycla* extract were not significantly different from their levels in the normal (negative control) group.

Figure 3 shows that similar results were detected on treatment of the formalin administered group. The dose dependent anti-inflammatory effect of *Phyllostachys heterocycla* was also pronounced in this model, where the serum levels of TNF- α , IL-1 β and IL-6 were significantly lower in the group treated by the higher dose (500 mg/kg) compared to the group treated by 250 mg/kg. Levels of the four inflammatory markers in the group treated by 500 mg/kg of *Phyllostachys heterocycla* were not significantly different from those recorded in the normal group.

Inflammation is a protective response of the living organism against infections and tissue injury. Inflammation involves a series of reactions, including vasodilation and recruitment of immune cells and plasma proteins to the affected site. The transcription factor NF- κ B is a central mediator of pro-inflammatory responses (**Lawrence, 2009**). It acts through targeting genes that are involved in the development and progression of inflammation, and mediates the induction of synthesis of pro-inflammatory cytokines in macrophages, including TNF- α , IL-1 β , and IL-6. Anti-inflammatory therapies targeting the NF- κ B pathway are among the most effective strategies of reducing inflammation (**Liu et al., 2017**).

TNF- α is a pivotal pro-inflammatory cytokine that has an important function in the regulation of the immune system during inflammation (**Baud et al., 2001**). It was proven to have a role in vasodilatation that accompanies inflammation through stimulating inducible nitric oxide synthase (iNOS) expression (**Sanders et al., 2001**), and stimulation of cyclooxygenase-2 (COX-2), leading to increased expression of the vasodilatory prostacyclin (PGI₂) (**Mark et al., 2001**). TNF- α also contributes via several mechanisms to the development of edema that is characteristic of

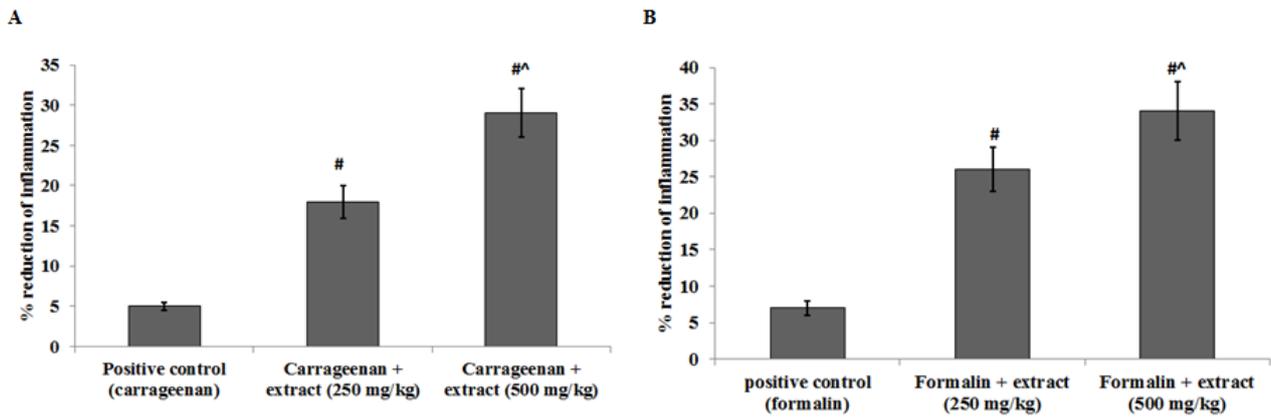


Figure 1: The percentage reduction of inflammation (paw thickness) caused by treatment with the crude extract of *Phyllostachys heterocycle* in: (A) carrageenan-induced, and (B) formalin-induced models. Data are represented as mean \pm SD. (n=8). Comparisons were performed by ANOVA followed by Bonferroni's post-hoc test. # significantly different compared to the positive control group. ^ significantly different compared to the group treated with 250 mg/kg of the extract. Differences were considered statistically significant at $P < 0.05$.

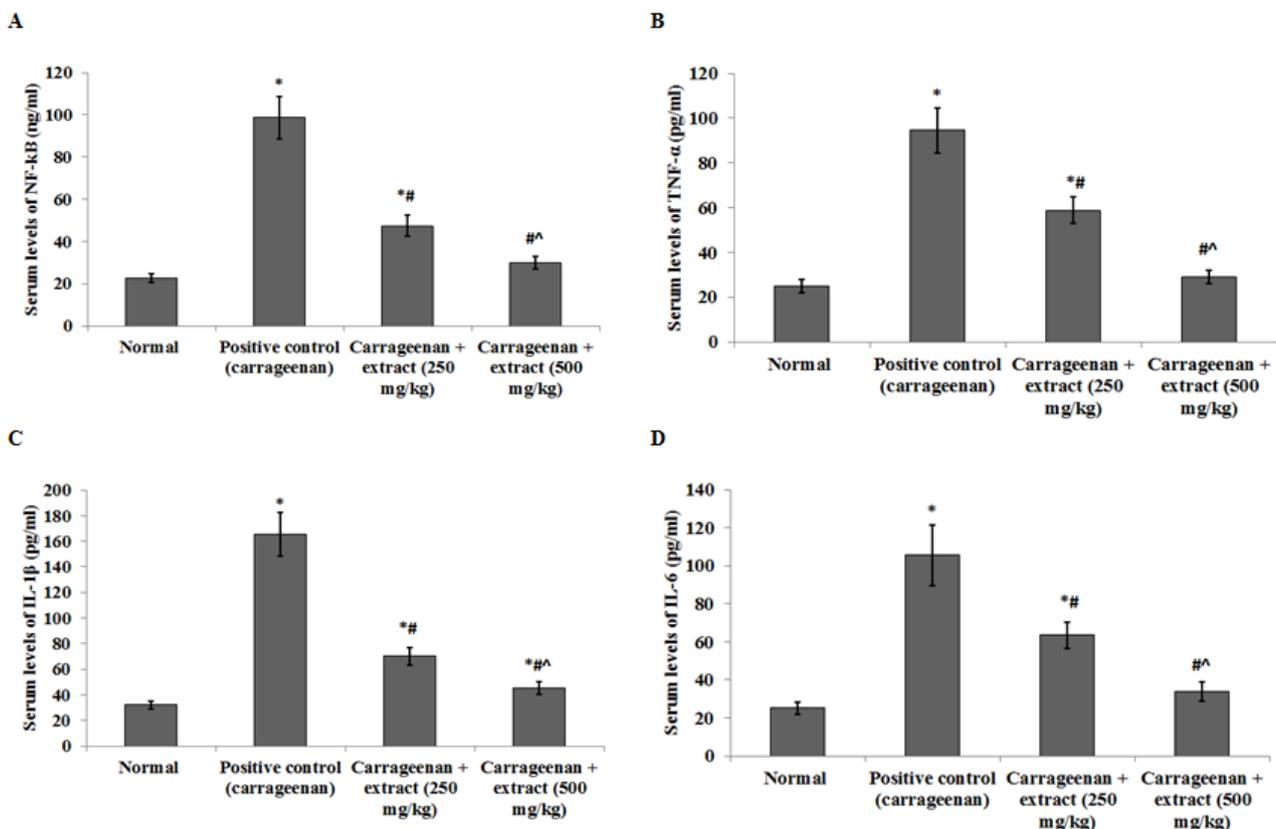


Figure 2: The effect of treatment with *Phyllostachys heterocycle* on the serum levels of (A) NF-κB, (B) TNF-α, (C) IL-1β and (D) IL-6 in carrageenan-induced model of inflammation. Data are represented as mean \pm SD. (n =8). Comparisons were performed by ANOVA followed by Bonferroni's post-hoc test. * significantly different compared to the normal (negative control) group. # significantly different compared to the positive control group. ^ significantly different compared to the group treated with 250 mg/kg of the extract. Differences were considered statistically significant at $P < 0.05$.

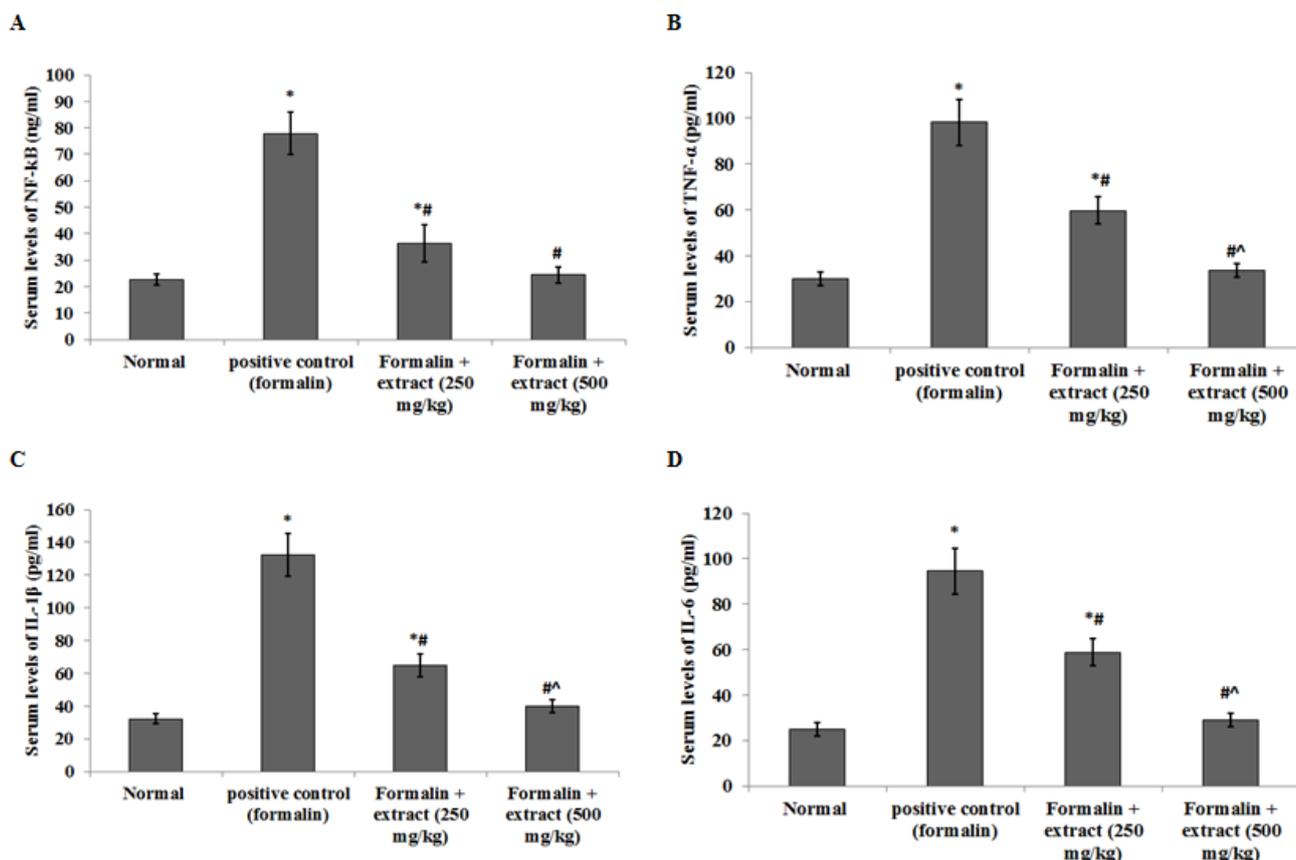


Figure 3: The effect of treatment with *Phyllostachys heterocycla* on the serum levels of (A) NF-κB, (B) TNF-α, (C) IL-1β and (D) IL-6 in formalin-induced model of inflammation. Data are represented as mean ± SD. (n=8). Comparisons were performed by ANOVA followed by Bonferroni's post-hoc test. * significantly different compared to the normal (negative control) group. # significantly different compared to the positive control group. ^ significantly different compared to the group treated with 250 mg/kg of the extract. Differences were considered statistically significant at $P < 0.05$.

inflammation (Zelová & Hošek, 2013). Epithelial cell TNF receptor surface expression was reported to be enhanced by IL-1β (Saperstein et al., 2009). TNF-α and IL-1β participate to the activation of the transcription factors that mediate the production of IL-6 (Tanaka et al., 2014). IL-1β and IL-6 are major pro-inflammatory cytokines that are produced by macrophages and monocytes at the inflammation site and play a key role in the acute phase response (Gabay, 2006). IL-6 is the chief stimulator of the production of most acute phase proteins such as C-reactive protein (CRP) and fibrinogen (Heinrich et al., 1990).

It is worthy to mention that the compounds previously isolated from *Phyllostachys heterocycla* possess anti-inflammatory activities which justify the results obtained upon examination of the crude extract (Figure 4). For example, Stigmast-4-en-3-one (2) exhibited anti-inflammatory activity through their nitric oxide (NO) inhibitory effect (Tewtrakul

et al., 2010). β-sitosterol (5) showed potent anti-inflammatory activity in specific and non-specific types of acute inflammation in rodents (Paniagua-Pérez et al., 2017). (6'-O-palmitoyl)-sitosterol-3-O-β-d-glucoside (6) showed in-vivo and in-vitro concentration dependent anti-inflammatory activity (Hernández-Valle et al., 2014). Moreover, β-sitosterol-3-O-β-d-glucoside (7) was proven to have anti-inflammatory activity through inhibiting the action and secretion of inflammatory mediators and elements (Choi et al., 2012).

4. Conclusion:

Examination for the anti-inflammatory activity of crude methanolic extract of *Phyllostachys heterocycla* in rats using two different models of paw inflammation showed a dose-dependent reduction of inflammation in both models. The low dose (250 mg/kg) decreased the inflammation in both the carrageenan-induced and formalin-induced

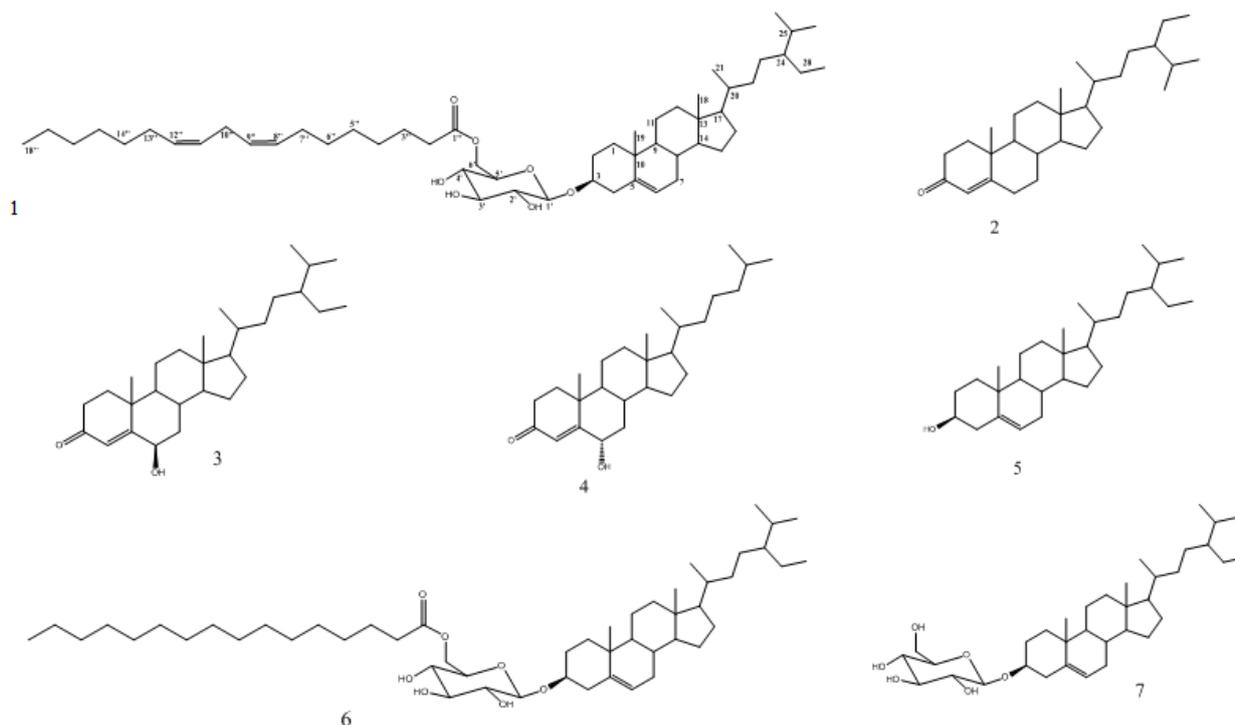


Figure 4: Compounds previously isolated from *Phyllostachys heterocycla* and reported to possess anti-inflammatory activity [6'-O-octadeca-8'',11''-dienoyl)-sitosterol-3-O- β -d-glucoside (1), Stigmast-4-en-3-one (2), 6-Hydroxy-24-ethylcholest-4-en-3-one (3), 6-Hydroxycholest-4-en-3-one (4), β -sitosterol (5), 6'-O-palmitoyl)-sitosterol-3-O- β -d-glucoside (6), β -sitosterol-3-O- β -d-glucoside (7)] (Abdelhameed et al., 2020).

models by 18% and 26%, respectively, while the high dose (500 mg/kg) caused a more pronounced decrease of paw thickness in both the carrageenan model by 29% and the formalin model by 34%. Reduction in paw thickness was accompanied by a significant decrease in the levels of the serum levels of NF- κ B, TNF- α , IL-1 β and IL-6 inflammatory markers. The high dose (500 mg/kg) decreased the serum levels of all markers effectively in both models of inflammation. This preliminary study suggests strong anti-inflammatory properties of *Phyllostachys heterocycle* extract. Further studies are required to confirm this effect and to investigate the major mechanisms that mediate this anti-inflammatory activity.

5. Conflict of interest:

The authors report no declaration of conflict of interest.

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