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Original Research Article

Anti-arthritic effect of total anthraquinone from Polygonum cuspidatum on type II collagen-induced arthritis in rats

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Abstract

Purpose: *To study the anti-arthritic effect of total anthraquinone from Polygonum cuspidatum (TAPC) on type II collagen-induced arthritis (CIA) in rats, and to investigate the underlying mechanism(s).*
Methods: *CIA rats were prepared and treated orally with TAPC at doses of 50, 100 and 200 mg/kg/day, for 24 days. Paw volume and arthritis score were measured prior to TAPC treatment, and subsequently at 3-day intervals on days 3, 6, 9, 12, 15, 18, 21 and 24. Serum levels of TNF-α, IL-6 and IL-17 were determined by enzyme-linked immunosorbent assay (ELISA), while synovial tissue TNF-α, IL-6 and IL-17mRNA expressions were assayed by real time-polymerase chain reaction (RT-PCR). Thymus and spleen indices were also determined.*
Results: *TAPC (50, 100 and 200 mg/kg) significantly alleviated paw swelling ($p < 0.05$), arthritis scores ($p < 0.05$) and thymus and spleen indices ($p < 0.05$) of CIA rats, when compared with the control rats. In addition, TAPC significantly decreased serum levels of the pro-inflammatory cytokines TNF-α, IL-6 and IL-17 ($p < 0.01$); and down-regulated their mRNA expressions in synovial tissues ($p < 0.01$).*
Conclusion: *These results suggest that TAPC exerts good anti-arthritic activity in rats, most probably via suppression of inflammatory responses.*

Keywords: *Polygonum cuspidatum, Anthraquinone, Type II collagen-induced arthritis, Pro-inflammatory cytokines*

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INTRODUCTION

Rheumatoidarthritis (RA) is a common destructive chronic systemic autoimmune disease of the joints [1,2]. It usually results in chronic proliferative synovitis and infiltration of inflammatory cells into the synovial tissue of joints, causing joint swelling, destruction of cartilage and bone, and disability [3,4]. In addition, RA leads to extra cares and economic burdens on the family of patients [5]. It's reported that over 0.5 % human beings in the world are suffered from the disease of RA [6,7]. Currently, drugs used for treating RA include non-steroidal anti‐inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs, glucocorticoids and biological drugs [8]. However, these drugs only bring about momentarily amelioration of the RA symptoms rather than radical cure. Moreover, long term use of these medications is expensive and toxic [8,9]. Consequently, there is need to find novel therapeutic approaches against RA.

Polygonum cuspidatum, a traditional Chinese medicine (TCM), has been used for treating inflammatory diseases for thousands of years [10,11]. Extensive reports have demonstrated that anthraquinones is the most important active

constituents of *P. cuspidatum* [12,13]. In addition, previous reports indicated that extracts of *P. cuspidatum* exerted significant anti-arthritic effect in rats, which effect was thought to be due to their anthraquinone contents [14]. However, to date, there are no studies on the anti-arthritic potential of active phytochemical constituents of *P. cuspidatum.* Consequently, the present investigation was aimed at investigating the anti-arthritic effect of total anthraquinones from *P. cuspidatum* (TAPC) against type II collagen-induced arthritis (CIA) in rats, and the mechanism(s) involved.

EXPERIMENTAL

Chemicals and materials

Total anthraquinones of *P. cuspidatum* (TAPC) were obtained from the Hunan Nutra Max Inc. (Changsha, China). Chicken type II collagen (CII), methotrexate (MTX), and complete Freund's adjuvant (CFA) were products of Sigma Co. (Shanghai, China). Rat tumor necrosis factor (TNF)-α, interleukin (IL)-6 and rat IL-17 ELISA kits were products of Abcam Co. (Cambridge, UK). All other chemicals used in this study were of analytical reagent grade.

Preparation of CIA rat model and experimental protocols

An arthritic rat model was established using type II collagen (CII). All the animal experimental protocols were according to the international standard protocols for the use of laboratory animals [15], and were approved by Animal Care and Use Committee of Heping Hospital Affiliated to Changzhi Medical College (approval ref no. KY-2016A-001).

Sixty rats were randomly divided into six groups (n=10): normal rats treated with saline alone (10 mL/kg); CIA control rats (arthritic rats treated with saline (10 mL/kg); MTX rats (CIA rats treated with 2 mg MTX/kg, 3 times a week); and CIA rats treated with TAPC at doses of 20, 100 and 200 mg/kg. The CIA rats were prepared as reported previously [2] with minor modifications. Type II collagen (CII) was dissolved in 0.1 mM acetic acid (4 mg/mL), and emulsified with an equal volume of CFA. Then, the rats were initially immunized by subcutaneously injection of the CII-CFA emulsion into the tail root (100 µL/rat). After 7 days, the rats were secondarily immunized by subcutaneous injection of the CII-CFA emulsion (100 µL/rat).

After 20 days of the initial CII immunization, the rats were orally administered saline, MTX and different doses of TAP (50, 100 and 200 mg/kg). During the observation period, the paw volumes of the rats were measured at the start, and then on days 3, 6, 9, 12, 15, 18, 21 and 24. Moreover, the arthritis scores were determined at the start, and subsequently at 3-day intervals on days 3, 6, 9, 12, 15, 18, 21 and 24. Arthritis was scored according to the ordinal rules [2], i.e., 0 = normal, 1 = obvious swelling or erythema observed in one joint, 2 = obvious swelling or erythema observed in two joints, 3 = obvious swelling or erythema observed in three joints, and 4 = three joints affected plus maximal erythema and swelling. After 24 days of treatment, the rats were weighed, and blood samples were collected through orbital blood sampling. Then the rats were sacrificed by decapitation, and the thymus and spleen were isolated for determination of their visceral indices (ratio of thymus or spleen wet weight to body weight). Synovial tissues were collected for western blot assays.

Determination of serum levels of TNF-α, IL-6 and IL-17

Blood samples were allowed to clot for 1 h at room temperature, and centrifuged for 15 min at 1800 g to obtain sera. Serum levels of TNF-α, IL-6 and IL-17 were determined by commercial ELISA kits according to manufacturer's instructions.

Determination of TNF-α, IL-6 and IL-17 mRNA expressions in synovial tissues

Synovial tissue samples were homogenized in liquid nitrogen and the total RNA was isolated by RNAiso Plus kits (TaKaRa, Tokyo, Japan). Then, cDNA was synthesized by reverse transcription with the PrimeScript™ RT reagent Kits (TaKaRa, Tokyo, Japan). Then, cDNAs were amplified by PCR assays with SYBR Green Mix kits (Bio-Rad, Shanghai, China) and the bands were detected by real-time PCR Detection System (Bio-Rad, Shanghai, China). All the primers used in this experiment were synthesized by Sangon Biotech (Shanghai, China Table 1). The relative mRNA expressions of TNF-α, IL-6 and IL-17 in synovial tissues were determined by $2^{-\Delta\Delta CT}$ relative quantitative analysis.

Statistical analysis

Data are expressed as mean ± SD and were evaluated using one-way ANOVA followed by Dunnett's multiple comparisons. SPSS software (SPSS for Windows 19.0; IBM Corp., Armonk, NY, USA) was used for the analysis, and statistical significance was set at $p < 0.05$.

Table 1: Primers used for real-time PCR

Gene name	Primer sequence		Reference
TNF-α	F:	5′-CAGGTTCTGTCCCTTTCACTCACT-	[16]
	R:	5′-GTTCAGTAGACAGAAGAGCGTGGT-	
IL-6	F:	5′-TGGAGTACCATAGCTACCTGGAGT-	[16]
	R:	5′-TCCTTAGCCACTCCTTCTGTGACT-	
IL-17	F:	5′-ATGAGTCCAGGGAGAG-3′	[17]
	R:	5′-TTAGGCTGCCTGGCGG-3′	
β-actin	F:	5′-GGGAAATCGTGCGTGACATCAAAG-	[16]
	R:	5′-CATACCCAAGAAGGAAGGCTGGAA-	

RESULTS

TAPC decreases paw swelling and arthritis scores in CIA rats

The CIA rats showed obvious symptoms of RA, such as paw swelling and higher arthritis scores ($p < 0.01$), when compared with normal, control rats (Figures 1 & 2). These CIA-induced RA symptoms were significantly mitigated by administration of MTX ($p < 0.01$). In addition, TAPC treatment at doses of 50, 100 and 200 mg/kg significantly and dose-dependently decreased the arthritis scores ($p < 0.01$) as well as paw swelling ($p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively) in CIA rats, when compared with untreated CIA rats. All these results suggested that TAPC might possess notable therapeutic effect for treating RA.

TAPC decreases thymus and spleen indices in CIA rats

As can be seen from Fig. 3, thymus and spleen indices of control rats (CIA rats) were significantly higher ($p < 0.01$) than those of normal rats. After treatment with MTX (2 mg/kg, 3 times a week), both the thymus and the spleen indices were decreased ($p < 0.01$), when compared with those of the control rats. Similarly, TAPC treatment at the dose of 200 mg/kg significantly reduced the thymus and spleen indices relative to those of the control rats ($p < 0.01$). TAPC administration at a dose of 100 mg/kg significantly decreased the thymus index ($p < 0.01$), when compared to the control rats.

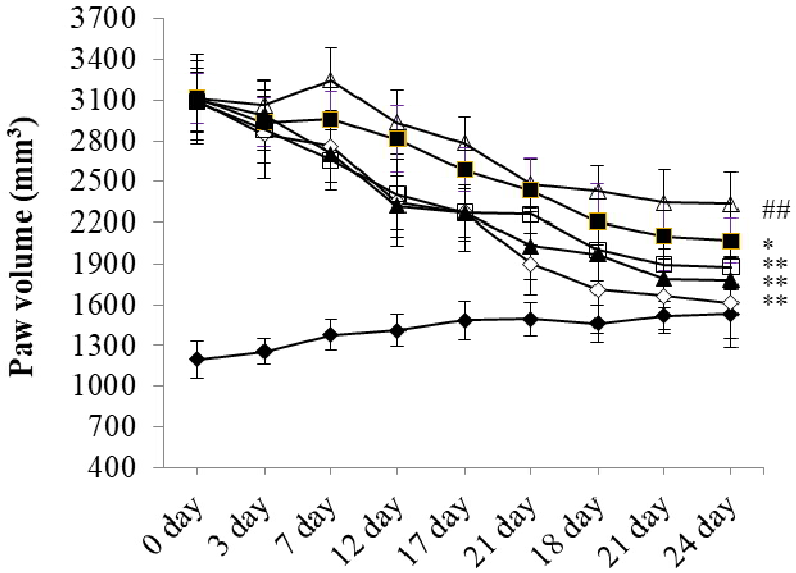

Figure 1: Effect of TAPC on paw volume changes of CIA rats. ***Note:*** Cont: control rats. Norm: normal rats. Data are presented as the mean ± SD (n = 10). $^{\#\#}p < 0.01$, *vs.* Norm; $^{*}p < 0.05$, $^{**}p < 0.01$, *vs.* Cont. Day 0 means day 20 after initial immunization. **Key:** ◆:Normal, △: Cont, ◇:MTX, ■:50mg/kg, □:100mg/kg,▲:200mg/kg

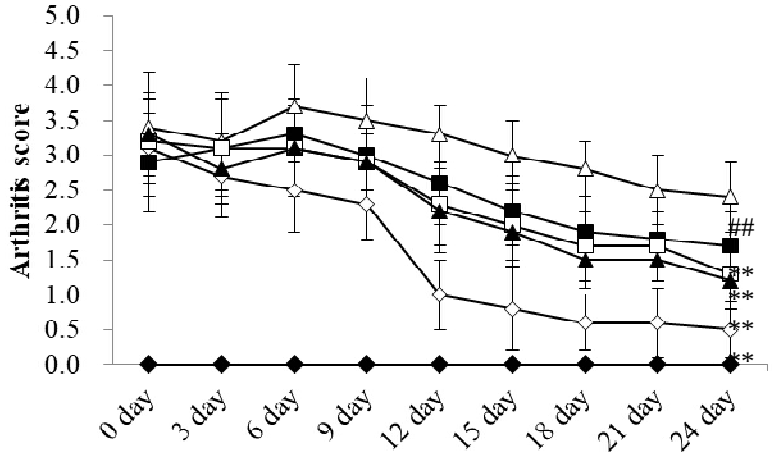

Figure 2: Effect of TAPC on arthritis score changes of CIA rats. ***Note:*** Cont: control rats. Norm: normal rats. Data are presented as the mean ± SD (n = 10). $^{##}p < 0.01$, *vs.* Norm; $^{*}p < 0.05$, $^{**}p < 0.01$, *vs.* Cont. Day 0 means day 20 after initial immunization. **Key:** ◆:Normal, △: Cont, ◇:MTX, ■:50mg/kg, □:100mg/kg,▲:200mg/kg

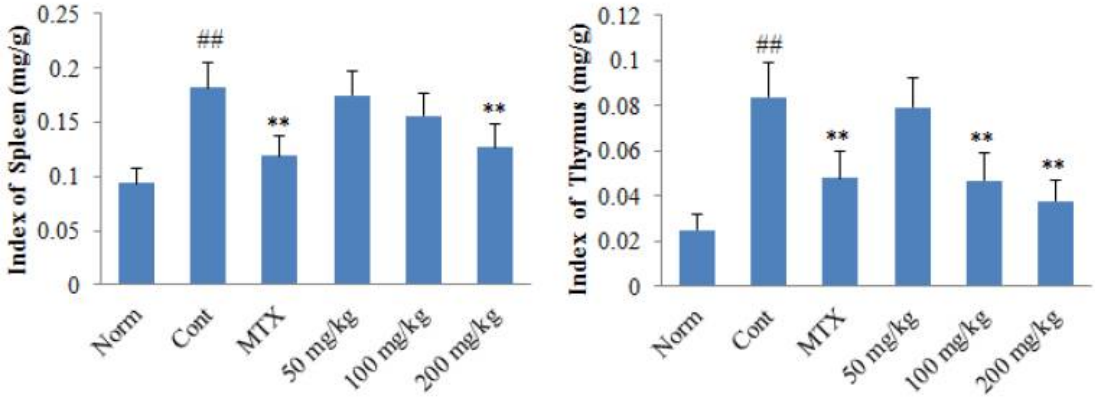

Figure 3: Effects of TAPC on thymus and spleen index in CIA rats. ***Note:*** Cont: control rats. Norm: normal rats. Data are presented as mean ± SD (n = 10). $^{##}p < 0.01$, *vs.* Norm; $^{*}p < 0.05$, $^{**}p < 0.01$, *vs.* Cont

TAPC decreased serum levels of TNF-α, IL-6 and IL-17 in CIA rats

Serum levels of TNF-α, IL-6 and IL-17 in untreated CIA rats were significantly higher than their corresponding levels in normal rats ($p < 0.01$). In addition, the levels of the three pro-inflammatory cytokines were significantly reduced in serum of rats treated with MTX. Interestingly, TAPC brought about significant and dose-dependent decreases in the serum levels of the pro-inflammatory cytokines TNF-α and IL-17 ($p < 0.01$), when compared to the untreated CIA rats. Furthermore, TAPC at doses of 100 and 200 mg/kg significantly decrease serum levels of IL-6 ($p < 0.01$), relative to the control, untreated CIA rats.

TAPC down-regulated TNF-α, IL-6 and IL-17 mRNA expressions in synovial tissues of CIA rats

As shown in Figure 5, compared to normal rats, mRNA expressions of the three cytokines were significantly elevated in the untreated CIA rats ($p < 0.01$). However, importantly, the present results also demonstrated that TTAPC treatment (50, 100 and 200 mg/kg) significantly and dose-dependently down-regulated the expressions of the mRNA expressions of TNF-α, IL-6 and IL-17 mRNAs in synovial tissues ($p < 0.01$), when compared with the control rats.

DISCUSSION

It has been reported that herbal medicines are good potential sources of novel anti-inflammatory agents which could be used for treating RA [2,18]. In the present work, the anti-arthritic activities of TAPC in a CIA rat model were studied for the first time, and the results demonstrate that TAPC possesses promising anti-arthritic potential via inhibition of inflammatory responses.

Increasing amount of evidence have demonstrated that RA is a typical immune mediated chronic inflammatory disease [19,20].

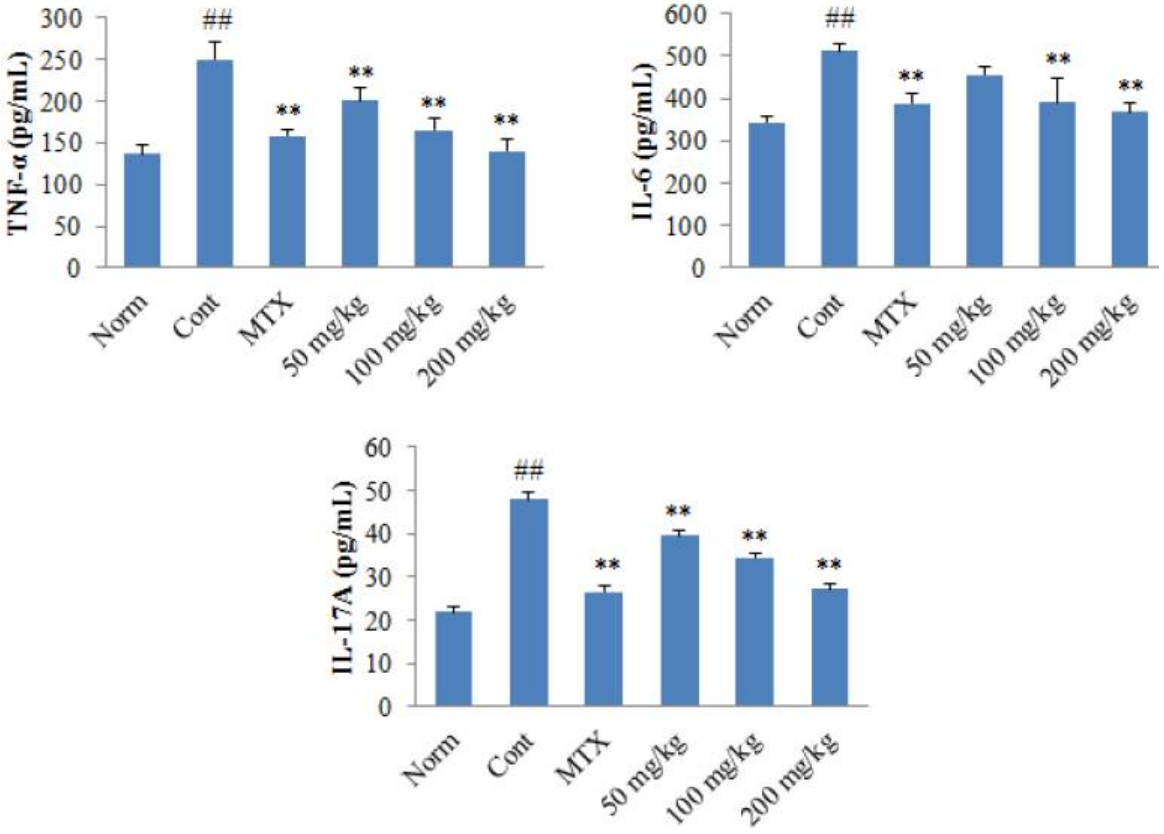

Figure 4: Effect of TAPC on serum levels of TNF-α, IL-6 and IL-17 in CIA rats. ***Note:*** Cont: control rats. Norm: normal rats. Data are presented as mean ± SD (n = 10). $^{\#\#}p < 0.01$, *vs.* Norm; $^{*}p < 0.05$, $^{**}p < 0.01$, *vs.* Cont

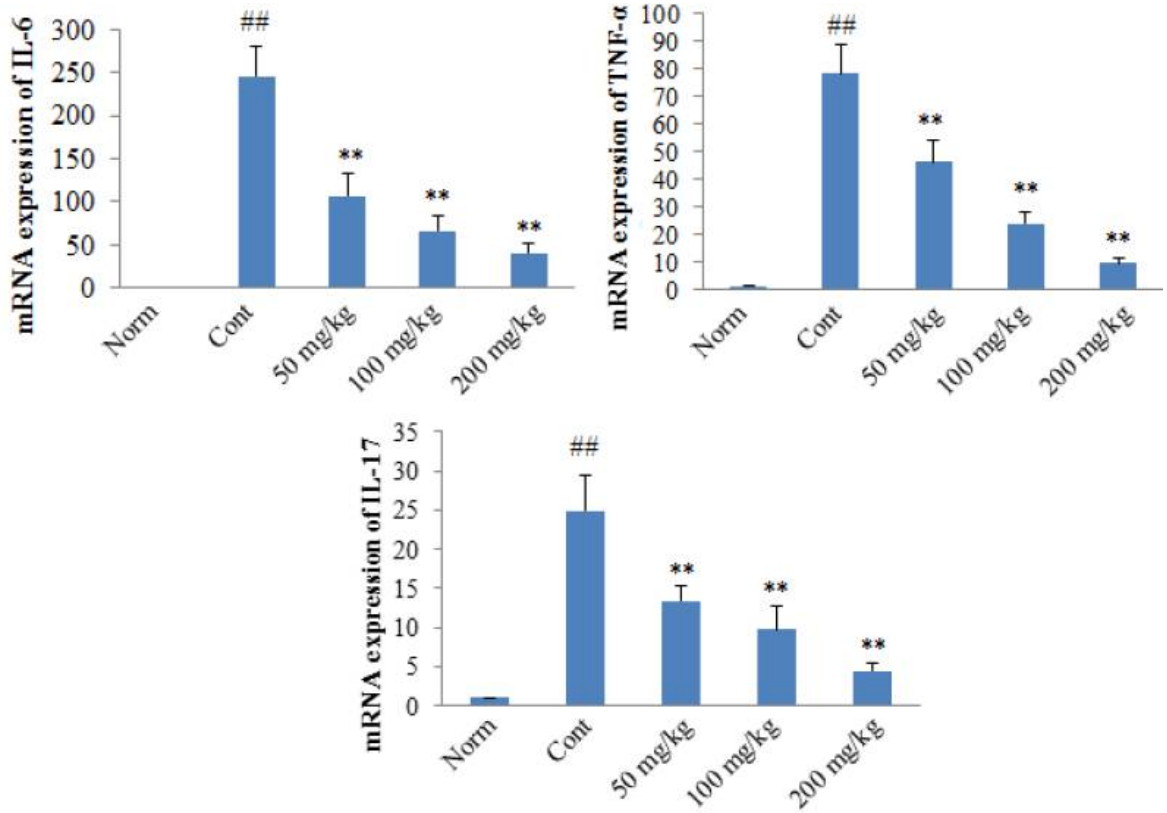

Figure 5: Effects of TAPC on mRNA expressions of TNF-α, IL-6 and IL-17in synovial tissues of CIA rats. ***Note:*** Cont: control rats. Norm: normal rats. Data are presented as mean ± SD (n = 10). $^{\#\#}p< 0.01$, *vs.* Norm; $^{*}p < 0.05$, $^{**}p < 0.01$, *vs.* Cont

Two mature animal models for investigation of RA have been developed. These are type II collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AIA) models [2,21]. Studies have demonstrated that CIA is a good RA animal model with immunological and pathological characteristics similar to those of human RA patients [21,22]. In the present study, the CIA rat model was successfully established, and used for evaluating the anti-arthritic effects of TAPC. The results obtained revealed that TAPC treatment effectively alleviated the CIA-induced paw swelling and high arthritis scores in the rats.
It is well known that the control of inflammatory responses is beneficial for inhibiting the development of RA [2,19,20]. The results of the present study indicate that TAPC can relieve inflammatory reactions in CIA rats, as evidenced by alleviation of paw swelling and decreases in arthritis scores. The present study also determined the serum levels of pro-inflammatory cytokines TNF-α, IL-6 and IL-17.These cytokines stimulate inflammatory reactions in arthritic joints, and have been reported to be the potential targets for the effective treatment of RA [1,2,23]. Treatment with TAPC effectively decreased the serum levels of these three cytokines in the CIA rats. Consistent with this finding, TAPC also significantly and dose-dependently down-regulated expressions of TNF-α, IL-6 and IL-17 mRNAs in synovial tissues of the CIA rats.

CONCLUSION

The results of the present investigation indicate that TAPC possesses good anti-arthritic properties in rats, through a mechanism involving suppression of inflammatory responses.

DECLARATIONS

Acknowledgement

None.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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