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Full Length Research Paper

Tomato leaves methanol extract possesses anti-inflammatory activity via inhibition of lipopolysacharide (LPS)-induced prostaglandin (PGE_2)

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Recently, the leaves of tomato plant that contained several active compounds including alkaloid, steroid and flavanoid has been used for the treatment of variety of diseases and as anti-cancer, anti-oxidant and anti-gout. Although, a number of pharmacological properties have already been demonstrated, the anti-inflammatory effect of tomato leaves and its associated molecular mechanisms have not yet been fully investigated. In this study, in order to observe the anti-inflammatory action of *Solanum lycopersicum* extract on lipopolysaccharide (LPS)-stimulated macrophages, its inhibitory and inflammation activity was investigated by observing the prostaglandin E_2 (PGE_2) production using PGE_2 enzyme immunometric assay kit. Results show that the tomato leaves extract reduced the activity of inflammatory mediators (PGE_2) which plays a central role in inflammatory activity. At the highest concentration (100 µg/ml) of tomato leaves extract tested, the PGE_2 production was reduced (37.41%) as compared to the untreated. The cyclooxygenase-2 (COX-2) gene expression also reduced following increase in the extract concentration. Hence, this present study may support the potential use of leaves of *Solanum lycopersicum* extract in the treatment of inflammatory related disease through the inhibition of PGE_2 released.

Key words: Anti-inflammatory mediator (PGE_2), *Solanum lycopersicum*, lipopolysaccharide (LPS), macrophages cells RAW264.7, immunometric assay kit.

INTRODUCTION

Inflammation is an essential aspect of host response that leads to infection and injury, and is required to maintain healthy state against bacterial and viral infections. However, excessive or aberrant inflammation contributes to many acute and chronic human diseases (Serha and Savill, 2005). However, inflammatory response is characterized by the abundant productions of prostaglandin E_2 (PGE_2) and thus, these pro-inflammatory mediators are important anti-inflammatory targets (Lawrence et al., 2002). This mechanism is an immunological response following bacterial infection and is primarily mediated by phagocytes macrophages. Prostaglandins are a group of biologically active compounds that play major roles in human physiology in both health and diseases. They function in many different ways and in all major organs. The rate-limiting enzyme in the synthesis of PGE_2 is cyclooxygenases (COX). Two main isoforms of COX have been described: COX-1 and COX-2. COX-1 is expressed constitutively in most tissues and is responsible for the homeostatic production of PGE_2. In contrast,

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Abbreviations: LPS, Lipopolysaccharide; **PEG_2,** prostaglandin E_2; **COX-2,** cyclooxygenase-2.

COX-2 is induced by several stimuli, including growth factors, mutagens, pro-inflammatory cytokines and tumor promoters. Its uncontrolled activity is thought to play an important role in the pathogenesis of many chronic inflammatory diseases (Harris et al., 2002). Besides, subsequent investigations indicated that over expression of COX-2 is frequently found in many cancers including colon, lung, breast, pancreas and head and neck cancers (Brochers et al., 2000), and is usually associated with poor prognosis and short survival (Indra Dharmu et al., 2007). It is indicated that treatment with selective COX-2 inhibitors may reduce the risk of Alzheimer's (Giovannini et al., 2003) and Parkinson's diseases (Teismann et al., 2003) and may also be effective in the treatment of asthma (Profita et al., 2003). Based on these observations, it has been hypothesized that the suppression of PGE_2 production in macrophages could serve as the basis for developing potential anti-inflammatory drugs. Meanwhile, from previous studies, non-steroidal anti-inflammatory drugs (NSAIDs) that are mainly used in the treatment of pain and inflammation related to a large variety of pathologies have been prepared and marketed (Norton, 1998). These have been of immense help in the management of various inflammatory conditions like rheumatism, arthritis and breast pain. However, these drugs are known to provoke gastrointestinal irritation. This makes them widely unacceptable, especially in the elderly where the disease is more prevalent, hence the search for alternative anti-inflammatory drugs and medicines among the bounties of natural herbs. Hence, there is much hope in finding anti-inflammatory drugs from traditional and medicinal plants without side-effects. Moreover, in the face of rising cost of orthodox medicines, phytomedicinal treatment of diseases has become the order of the day in most parts of Africa, chiefly because of their ready affordability and availability, especially in the rural set-ups where the greater percentage of the people are poor and merely subsisting. Many plants in the Solanaceae family, such as tomatoes, potatoes and eggplant, possess steroidal alkaloids based on a C27 cholestane skeleton, such as tomatidine and solasodine. These compounds are essentially nitrogen analogues of steroid saponins such as diosgenin, which is a precursor of steroidal hormones and anti-inflammatory steroids (Norton, 1998). Steroidal alkaloids and their glycosides are known to possess a variety of biological activities, including antifungal and antibacterial actions (Steel and Drysdale, 1988). However, understanding of the anti-inflammatory effects of steroidal alkaloids is very limited. There is currently a strong interest in developing new anti-inflammatory agents from plants used in traditional medicine. Tomato (*Solanum lycopersicum)* has been used as flavoring agents in food, and beverages and as well as an alternative medicine to treat individuals with cancer, bronchitis, allergies and gout (Kashfi and Rigas, 2005). Due to these facts, until now, there are no studies on the action of *S. lycopersicum* especially its leaves, in anti-inflammatory responses. In the present study, the anti-inflammatory effects of tomato leaves extract were investigated using LPS- induced inflammation.

Meanwhile, *S. lycopersicum* is one of the world's major vegetables with a worldwide production of 126 million tons in 2005 (FAOSTAT, 2007). It is an excellent source of many nutrients and secondary metabolites that are important for human health: mineral water, vitamins C lycopene, flavanoids, phenolics and chlorophyll (Giovanelli and Paradiso, 2002). Besides, there are many studies on the tomato including as anti-cancer, anti-gout and antioxidant (lycopene) (Junichiro, 2006). Since *S. lycopersicum* has long been used for the treatment of variety of disease, the present study aimed to investigate the inhibitory activity of tomato leaves against PGE_2 using RAW264.7 macrophages cells and introduce a new potential of anti-inflammatory action of some other parts of the tomato plants. On the other hand, hopefully, the discovery of anti-inflammatory properties of tomato leaves could also lead to the development of a new generation of drugs that possess both chemotherapeutic and chemo preventative properties, which are safer and more effective without having consequences in the patients.

MATERIALS AND METODS

Phosphate buffer saline (PBS), lipopolysaccharides (LPS) from *Escherichia coli,* Dulbecco's modified eagles medium (DMEM), penicillin-streptomycin and fetal bovine serum (FBS) were purchased from Invitrogen (GibcoBRL, USA). PGE_2 enzyme immunometric assay (PGE-EIA) kit and Cytokine-specific DuoSet® ELISA Development system were purchased from R&D System, Minneapolis, MN, USA. Dimethylsulfoxide (DMSO), methanol and other reagents of analytical grade were obtained from Merck (Darmstadt, Germany).

Preparation of *S. lycopersicum* leaves extract

Fresh tomato leaves were collected from Cameron Highlands, Pahang, Malaysia and washed using tap water before drying at 40°C for one day in drying oven (Junichiro et al., 2006). The dried leaves were ground using a grinder machine to increase the surface area. The powdered leaves were then extracted using 82% methanol with 1:10 (w/v) ratio. The mixture was agitated at 22°C, 110 rpm for 24 h in shake flasks. The mixture was filtered with Whatman No.1 filter paper to collect the filtrate. Finally, the filtrate was concentrated in a water bath at 40°C. The extract was dissolved in 10% of culture-grade dimethylsulfoxide (DMSO; Sigma-Aldrich, St.Louis, MO, USA) (w/v) ratio for further use.

Cell cultures

Frozen RAW 264.7 murine macrophage cells (ATCC No: TIB-71) was thawed. Each cell type was inoculated into two T-75 flasks, and allowed to grow until 60 to 80% confluent prior to inoculation at 2×10^6 cells in a new T-75 flask. The cells were cultured in DMEM (Dulbecco's modified eagle's medium) (GIBCO, USA) supplemented with 5% heat-inactivated fetal bovine serum (FBS) (GIBCO, USA), 100 µg/ml streptomycin (GIBCO, USA), 100 U/ml penicillin (GIBCO, USA) (Indra Dharmu et al., 2007) at 37°C with 95% air

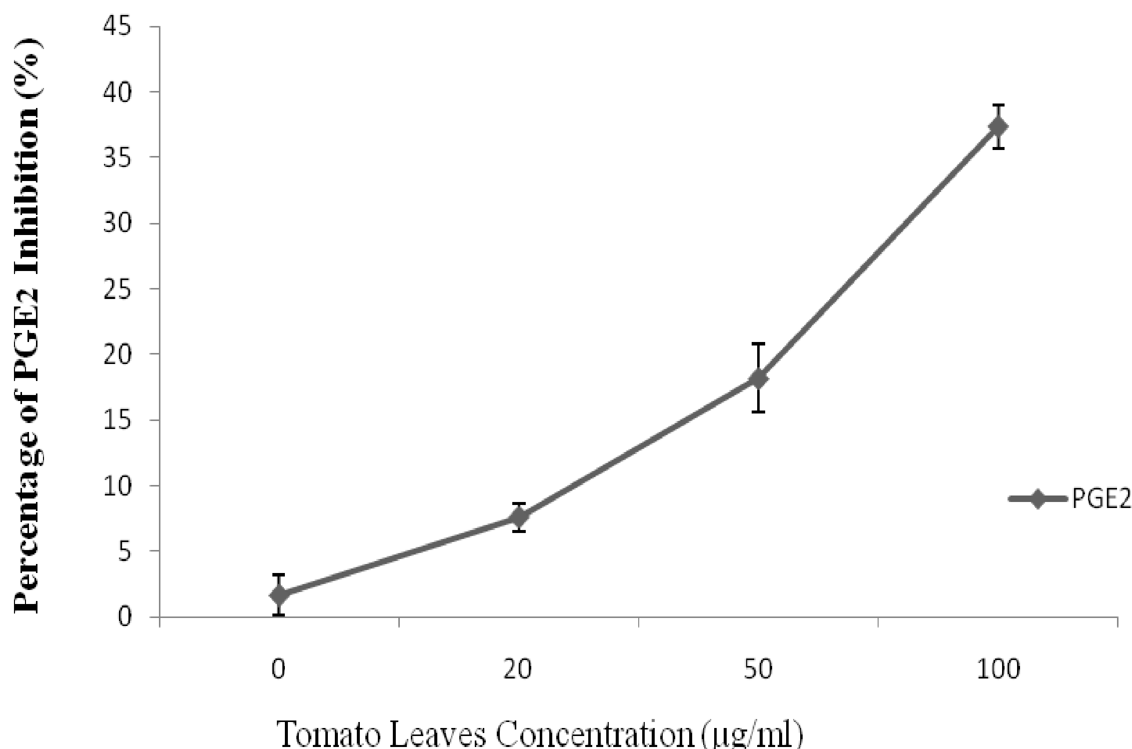

Figure 1. The percentage of PGE_2 inhibition by RAW 264.7 cell treated with different concentrations of tomato extract (0, 20, 50 and 100 µg/ml) and then treated with LPS (1 µg/ml). Three independent experiments were performed, and the data shown indicate the mean ± S.E.M (P<0.05).

and 5% CO_2 atmosphere.

Prostaglandin E2 (PGE2) assay

Assessment of PGE_2 synthesis was performed using a commercially available PGE_2 enzyme immunometric assay kit (PGE-EIA, R&D System, Minneapolis, MN, US). After RAW 264.7 cells were confluenced, cells were treated with the tomato leaves extract (0, 20, 50 and 100 µg/ml) for 30 min. Commercial bromelain (Maurer, 2001) and DMSO 10% were used as a positive and negative control, consecutively. Then, 1 µg/ml of LPS was added and cells were co-stimulated for 24 h. After LPS stimulation, PGE_2 synthesis was performed on the culture media through a PGE_2 enzyme immunometric assay (PGE-EIA) according to the manufacturer's instructions. Then, the inhibition levels of PGE_2 were determined by using the following formula:

$$\text{Percentage of inhibition } (\%) = \frac{A\text{-}B}{A\text{-}C} \times 100 \quad (1)$$

Where, A is LPS (+), sample (–); B isLPS (+), sample (+) and C is LPS (–), sample (–)

Real-time PCR

Total RNA was isolated using Trizol^{TM} (GibcoBRL) and reverse transcribed into $_C$DNA as per the manufacturer's instructions. Each transcript was identified using specific forward and reverse primers. GADPH expression was included as an internal housekeeping gene control. Ethidium bromide-stained PCR products were separated by electrophoresis on a 1% agarose gel in 1 x TAE and visualized by UV transiluminator. Primer sequences were as follows: GADPH (accession no. 001473632) as an internal control for PCR, 5'- GAC ATC ATA CTT GGC AGG-3' (forward), 5'-CTC GTG GAG TCT ACT GGT-3' (reverse). COX-2 (accession no.011198), 5'- ATG CTC CTG CTT GAG TAT GT-3' (forward), 5'- CAC TAC ATC CTG ACC CAC TT-3' (reverse); PCR reaction was performed at 94℃, 5 min for denaturation; 55℃, 1 min for annealing, and 72℃ for elongation and repeated for 35 cycles.

RESULTS AND DISCUSSION

Since PGE_2 is also known to be pro-inflammatory mediator in many different acute and chronic inflammatory diseases as well as in normal defense reactions, we also examined whether tomato leaves extract modulates PGE_2 production in LPS-activated macrophages. Figure 1 shows the percentage of PGE_2 inhibition when treated with different concentrations (0, 20, 50 and 100 µg/ml) of tomato leaves extract. From our observation, 100 µg/ml *S. lycopersicum* extract strongly inhibited PGE_2 production in the LPS-activated macrophages with 37.41 ± 0.71% inhibition. Figure 2 shows that the expression of COX-2 gene was reduced following the increase of *S. lycopersicum* extract concentration. This results suggest that the *S. lycopersicum* extracts targeted the inflammatory pathway at the gene level, thus, inhibited PGE_2 (inflammatory mediator) production. In general, prosta-

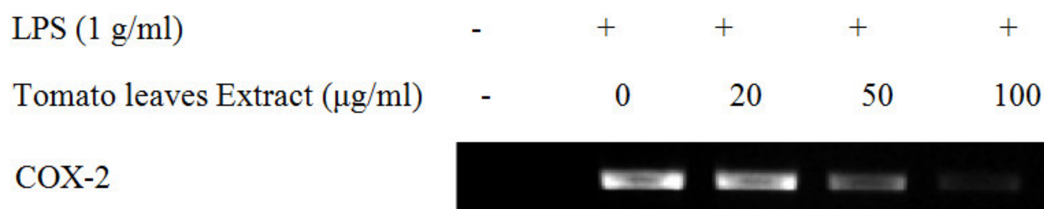

Figure 2. The inhibitions of products by reduction in the expression level of COX-2 gene. PCR product amplified from macrophage cell treated with different concentrations of leaves extract and LPS.

glandin E_2 (PGE_2) is considered as one of the strongest inflammatory mediators in inflammatory response. It was transformed from arachidonic acid via the cyclooxygenases 2 (COX-2) catalytic reactions. Nonsteroidal anti-inflammatory drugs (NSAIDS), which are used widely in current clinical practice, play their anti-inflammatory and analgesic role through the reduction of inflammatory mediator production including PGE_2 (Vogel, 1997).

Our study shows that leaves of *S. lycopersicum* could inhibit PGE_2 production in LPS-stimulated RAW264.7 cells, suggesting that the anti-inflammatory effect of tomato might be attributed to its inhibitive effect on COX-2. COX-2- selective inhibitors have similar anti-inflammatory properties with NSAIDS, but their gastrointestinal safety is significantly better than that of the latter, so it would be a better choice to use COX-2 selective inhibitors than NSAIDS in the reduction of pain and inflammation.

PGE_2 is produced by a wide variety of tissues and macrophages. However, the increasing amounts of PGE_2 cause several pathologic conditions which contributes to the development of many chronic inflammatory diseases, such as cardiovascular disease, cancer and rheumatoid arthritis (Vogel, 1997).

Macrophages are the first line of host defenses against inflammation and play important role in adaptive immune response (Verstovsek, 1992). Macrophages also exposed to stimulation agents such as bacterial lypopolysaccharides (LPS) release several inflammatory cytokines and other substances including COX-2. COX-2 is an inducible enzyme that is responsible for the synthesis of prostaglandins in the inflammation process. These enzymes are inhibited by several plant extract such as wogenin from *Scutellaria* radix (Chi et al., 2003) and apigenin (Liang et al., 1999).

Due to this problem, administrations of anti-inflammatory drugs that inhibit COX-2 activity have been shown by some investigators to be beneficial in preventing and treating these diseases (Bertolini and Ottani, 2002).

In the present study, we found that *S. lycopersicum* extract inhibited the PGE_2 production in LPS-stimulated RAW264.7 cells at the highest concentration of extract, which is 100 µg/ml. This study suggests that the inhibition of PGE_2 production by *S. lycopersicum* extract might be due to the suppression of COX-2 regulation during the activation of macrophages by LPS.

Conclusion

S. lycopersicum leaves methanol extract have an anti-inflammatory activity proved by the inhibition of LPS-induced inflammatory mediator (PGE2) and reduces the COX-2 gene expression.

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