the production of TNF-α by 41%, 25%, and 47%, respectively [Figure 2].

At compound level as well as at extract level, after 6 h of incubation, 0.74 ng/mL TNF-α was produced by blank RAW cells. The concentration of TNF-α in LPS-induced cells was 5.47 ng/mL, an increase of about 8 folds. The concentration of TNF-α was decreased more by treatment with selected compounds at higher concentration except 11. TNF-α production after treatment with 25 μg/mL of 11 was lower than that after treatment with 11 at higher concentrations (50 and 100 μg/mL). At the same concentration, most compounds resulted in lower concentration of TNF-α than that of treatment with EGCG, the positive control [Figure 3].

Inhibitory activity on interleukin-6 production

LPS treatment resulted in a significantly increased production of TL-6 (about 6 folds) compared to blank (non-LPS treatment) (IL-6 concentration of 502 and 88 pg/mL, respectively). Treatment with leaf extract, bark extract, and isolated compounds significantly suppressed IL-6 production in a dose-dependent manner, whereas treatment with positive control did not. Specifically, treatment with leaf extract, bark extract, and isolated compounds 8, 9, 10, and 11 at their highest concentration suppressed IL-6 production to 55%, 54%, 24%, 66%, 14%, and 33%, respectively [Figures 4 and 5].

DISCUSSION

Inflammation is a complex process regulated by a cascade of cytokines, growth factors, NO, and PGs by activated macrophages. NO is a short-lived molecule that has an important role in various physiologic processes. However, NO overproduction can activate nuclear factor-κB to induce the expression of pro-inflammatory mediators. It also promotes inflammation by increasing cyclic guanosine monophosphate, vascular permeability, and transmigration of inflammatory cells to inflammation sites.[1,6] NO toxicity increases considerably if it reacts with a superoxide radical and forms highly reactive peroxynitrite anion that can lead to oxidative damage and tissue injury.[17] Chronic generation of NO radical is associated with various cancers and inflammatory conditions, including diabetes mellitus and arthritis. Pro-inflammatory cytokines such as TNF-α and IL-6 evoke elevated levels of iNOS followed by a significant increase in the production of NO.[6]

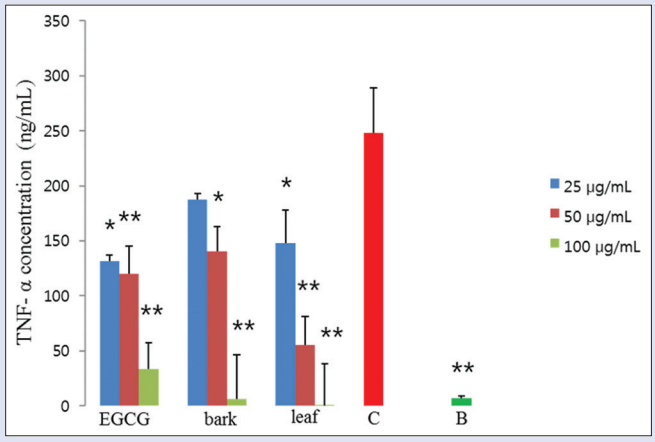

Figure 2: Inhibitory effect of extracts on tumor necrosis factor-α production in lipopolysaccharide-activated RAW macrophages. Epigallocatechin-3-gallate was used as a positive control. Data are presented as mean ± standard deviation of three experiments in triplicates. *$P < 0.05$, **$P < 0.01$, compared with the control group

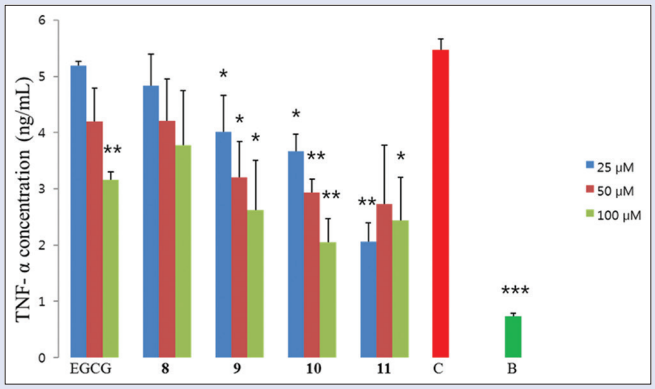

Figure 3: Inhibitory effect of selected compounds on tumor necrosis factor-α production in lipopolysaccharide-activated RAW macrophages. Epigallocatechin-3-gallate was used as a positive control. Data are presented as mean ± standard deviation of three experiments in triplicates. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with the control group

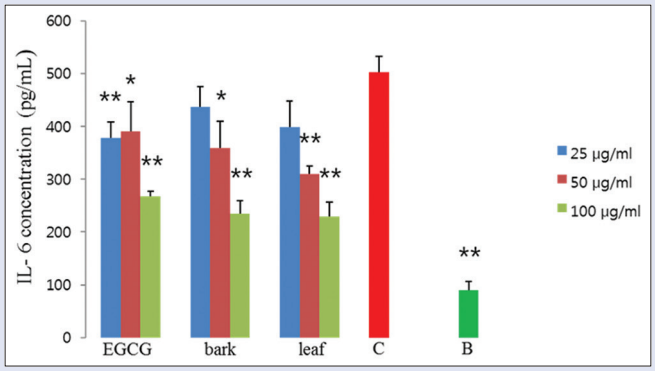

Figure 4: Inhibitory effect of extracts on interleukin-6 production in lipopolysaccharide-activated RAW macrophages. Epigallocatechin-3-gallate was used as a positive control. Data are presented as mean ± standard deviation of three experiments in triplicates. *$P < 0.05$, **$P < 0.01$, compared with the control group

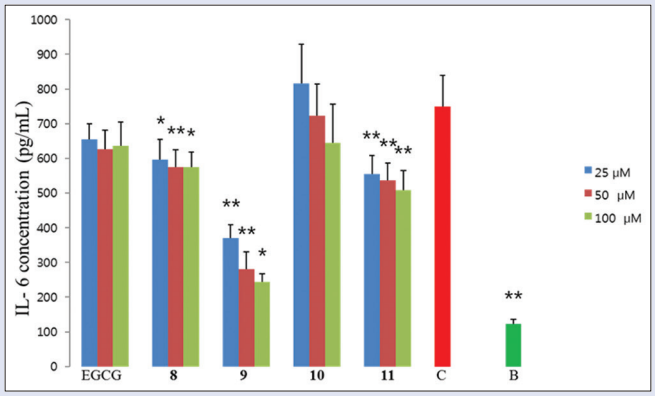

Figure 5: Inhibitory effect of selected compounds on interleukin-6 production in lipopolysaccharide-activated RAW macrophages. Epigallocatechin-3-gallate was used as a positive control. Data are presented as mean ± standard deviation of three experiments in triplicates. *$P < 0.05$, **$P < 0.01$, compared with the control group

Recently, inflammatory effects of MeOH twig extract of CL have been investigated both *in vitro* and *in vivo*. Results indicated that MeOH twig extract of CL effectively inhibited excessive NO production in transferrin-γ and LPS-stimulated mouse peritoneal macrophages without notable cytotoxicity. It also attenuated the expression of inflammatory mediators such as inducible NOS and cyclooxyganase-2 *in vivo*. The MeOH twig extract of CL also significantly suppressed paw edema induced by trypsin and carrageenan.[18] However, in our study, we focused on leaf extract of CL and isolated compounds from leaves. Isolation from bark extract was performed simultaneously with leaf extract to compare the anti-inflammatory effects of different parts of CL.

We performed studies at two levels: extract level and compound level. In terms of extract level, there were no significant differences in inhibitory activity on NO, TNF-α, or IL-6 production between leaf and bark extracts. Their cytokine production inhibitory activity was similar to that of the positive control. At high concentration, the extracts of CL had better cytokine production inhibitory activity than that of the positive control. At compound level, four compounds (8–11) significantly decreased NO production in LPS-stimulated RAW 246.7 cells. Their anti-inflammatory activity on LPS-stimulated expression of pro-inflammatory cytokines including TNF-α and IL-6 was also assessed. All compounds showed potent inhibition of TNF-α production. Among them, 11 showed the greatest inhibitory activity. For IL-6 inhibitory ability, 9 showed the greatest inhibitory activity. 10 was inactive in terms of inhibiting the production of IL-6, whereas 8 and 11 showed potent inhibitory effect on IL-6 production. Therefore, the anti-inflammatory ability presented by leaf extract of CL can be partly attributed to the presence of these compounds by decreasing the inflammatory response induced by LPS.

CONCLUSIONS

Our results indicated that ellagitannins (8–10) and flavonoids (11) were probably related to the anti-inflammatory activity of CL leaf extract, providing evidence of the anti-inflammatory property of CL. However, further researches are needed to elucidate the mechanisms involved in the anti-inflammatory activity of the isolated compounds and CL extracts.

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Conflicts of interest

There are no conflicts of interest.

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