Apigenin Acts as Anti-inflammatory Compound through Reducing IL-1β, IL-6 and TNF-α in LPS-induced Inflammation; in-vivo Study

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Research

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Abstract

Background: Consumption of herbal flavonoids instead of chemical drugs has increased in recent years due to fewer side effects and affordability. In this study, the effect of apigenin was investigated on inflammation induced by lipopolysaccharide in male rat's serum by measuring the pro-inflammatory cytokines, i.e., IL-1β, IL-6, and TNF-α.

Methods: 90 male Wistar rats weighing 200 ±2 grams were used and divided into control, sham (solvent), and positive control (dexamethasone 15 mg/kg. ip), and 3 experimental groups which received 5, 15 or 30 mg/kg of apigenin, intraperitoneally. In 30 minutes after interventions, lipopolysaccharide (LPS) [30 µg/kg. ip] was injected. Then, at 4, 12- and 24-hour intervals, rats were anesthetized, and blood samples were prepared intracardially. Samples were centrifuged, and serums were separated and stored at -80 ° C. Measurement of IL-1β, IL-6, and TNF-α were conducted by the enzyme-linked immunosorbent assay (ELISA) method. Data were analyzed using the SPSS software version 19.

Results: Pre-injection of apigenin at 5 mg/kg dosage were reduced TNF-α and IL-1β levels at 24-hours after LPS injection, compared to control (for both $P < 0.05$). Pre-injection of 15 mg/kg of apigenin was reduced IL-6 level at 24-hours after LPS injection ($P < 0.05$). Pre-injection of 30 mg/kg of apigenin were reduced TNF-α level at 4- ($P < 0.05$), 12- (*P <0.01*) and 24- (*P <0.01*) hours, IL-1β level at 24-hours (*P <0.01*), and IL-6 level at 4- (*P <0.05*) and 24- (*P <0.01*) hours after LPS injection.

Conclusions: Apigenin reduces proinflammatory cytokines in serum in acute inflammation induction. This impact is close to the dexamethasone effect as an anti-inflammatory steroid drug.

1. Introduction

Inflammation is a complex immune response to eliminating various pathophysiological statuses, such as pathogens, trauma, toxic compounds (1). Cytokines are small proteins that can affect both dual roles like pro- and anti-inflammatory. The majority of cytokines are produced by activated lymphocytes and macrophages, endothelial and epithelial cells. The expression of cytokine may be characterized by lipopolysaccharide (LPS), reactive oxygen species (ROS), microbial species, and other similar factors (2). Among all the cytokines associated with inflammation, more focus is on interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) (3), because their footprints could be seen in most inflammatory diseases, such as rheumatoid arthritis, atherosclerosis, metabolic syndrome, diabetes, chronic ulcerative colitis, neurodegenerative disorders and some forms of cancer (4, 5). Interleukin 6 (IL-6) is another important cytokine of the acute phase response, which triggers fever by synthesizing PGE2 in the hypothalamus (6). IL-6 has broad pro-inflammatory and anti-inflammatory roles (7). Major anti-inflammatory drugs, e.g., non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, induce various side effects, including gastrointestinal complication and systemic immunosuppression, respectively. In view of the above, there are various ongoing studies to find alternative therapies to treat inflammatory diseases (8).

Flavonoids are water-soluble polyphenols with established anti-cancerous, anti-oxidant, anti-bacterial, and anti-inflammatory effects, which protect mammals from cancer and other diseases through interacting in cellular mechanisms (9–11). Flavonoids inhibit inflammatory mediators such as ROS and nitric oxide (NO) and adjust the activity of the inflammatory-related enzyme, e.g., cyclooxygenases (COXs) and inducible nitric oxide synthase (iNOS), which modulate and reduce the production and expression of cytokines and transcription factors, such as NF-κB and AP-1 (12). Apigenin, as a flavonoid, is found in celery, chamomile, and parsley, while its anti-inflammatory and anti-oxidant properties are established in current studies (13, 14). Despite various studies, the exact cause of the of anti-inflammatory effect for apigenin have not yet been identified. In this study, we examined the anti-inflammatory effect of apigenin on LPS-induced inflammation (LPS increases the IL-1β, IL-6, and TNF-α cytokines, as major inflammatory cytokine profile) compared to dexamethasone in the animal model. Our aim was to introduce more details of the anti-inflammatory effect of apigenin.

2. Materials And Methods

2.1. Preparation of Materials and Animals

In this experimental study, 90 male Wistar rats (200 ± 2gr) were obtained from the Razi Institute (Karaj-Iran). Animals were kept in standard condition, including 12–12 h dark/light, sound, 22 ± 1°c temperature, and the ad-lab water and food. All experiments were conducted according to the policy of the Iranian Convention for the Protection of Vertebrate Animals used for Experimental Purposes, and the protocol was approved by the Ethics Committee of the Health School, Qazvin University of Medical Sciences Qazvin, Iran (IR.QUMS.REC.1395.179). LPS and apigenin (Sigma Aldrich-Germany), ketamine and xylazine (Alfasal Company-Dutch), dexamethasone (Hakim-Iran), ELISA kits (Uscan-USA) were obtained.

2.2. Pre-treatment and induction of inflammation

Initially, the rats were randomly divided into 6 groups ($n = 15$), i.e., control (no intervention), sham (intervention by a solvent; normal saline), positive control (intervention by 15 mg/kg of dexamethasone), and three experimental groups (5, 15 and 30 mg/kg of apigenin). All materials were diluted with normal saline and injected intraperitoneally (IP). After 30 minutes, LPS (30 µg/kg, i.p) was injected for inducing inflammation.

2.3. Sampling and Measurement of Inflammatory Factors

After 4, 12, and 24 hours passing from LPS injections, rats were anesthetized with IP injection of Ketamine and Xylazine (50 and 5 mg/kg, respectively), and the blood samples were prepared intracardially. The blood samples were centrifuged at 4000 rpm for 15 minutes. The serums were stored at -80 ° C until the measurement. The concentration of IL-1β, IL-6, and TNF-α was measured by ELISA as manufacturer protocol.

2.4. Data analysis

Data were analyzed using SPSS Ver.19 via ANOVA and Tukey's post hoc tests (the least significant difference was considered $P < 0.05$).

3. Results

In general, there was no significant difference between the control and sham (solvent) groups in intervals for measuring the pro-inflammatory cytokines. Therefore, for being easy of the data expression, the results of the sham group have not been considered.

3.1. Measurement of TNF-α

In 4 hours passing from LPS injections, 30 mg/kg of apigenin significantly decrease the level of TNF-α compared to the control group ($P < 0.05$), while other dosages of apigenin didn't significantly affect on TNF-α level. Also, in 12 hours passing from LPS injections, 30 mg/kg of apigenin significantly decrease the level of TNF-α compared to the control group ($P < 0.01$), while other dosages of apigenin didn't significantly affect on TNF-α level. Whiten 24 hours passing from LPS injections, 5 and 30 mg/kg of apigenin significantly decrease the level of TNF-α compared to the control group ($P < 0.05$ and $P < 0.01$, respectively), while 15 mg/kg of apigenin didn't significantly affect on TNF-α level. As a positive control, dexamethasone significantly decreases the TNF-α level compared to the control group in 4h-, 12- and 24h-hours passing from LPS injection ($P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively). (Fig. 1)

3.2. Measurement of IL-1β

Whiten 4- and 12-hours passing from LPS injections, no dosages of apigenin significantly decrease the level of IL-1β. In 24 hours passing from LPS injections, 5 and 30 mg/kg of apigenin significantly decrease the level of IL-1β compared to the control group ($P < 0.05$ and $P < 0.01$, respectively), while 15 mg/kg of apigenin didn't significantly affect IL-1β level. As a positive control, dexamethasone significantly decreases the IL-1β level compared to the control group in 4h-, 12- and 24h-hours passing from LPS injection ($P < 0.05$, $P < 0.001$, and $P < 0.01$, respectively). (Fig. 2)

3.3. Measurement of IL-6

In 4 hours passing from LPS injections, 30 mg/kg of apigenin significantly decrease the level of IL-6 compared to the control group ($P < 0.05$), while other dosages of apigenin didn't significantly affect on IL-6 level. Also, whiten 12 hours of passing from LPS injections, no dosages of apigenin significantly decrease the level of IL-6. Whiten 24 hours passing from LPS injections, 15 and 30 mg/kg of apigenin significantly decrease the level of IL-6 compared to the control group ($P < 0.05$ and $P < 0.01$, respectively), while 5 mg/kg apigenin did not significantly affect TNF-α level. As a positive control, dexamethasone significantly decreases the TNF-α level compared to the control group in 4h-, 12- and 24h-hours passing from LPS injection ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively). (Fig. 3)

4. Discussion

The mechanisms of the anti-inflammatory effect of apigenin are persist unclearly. We studied the effects of apigenin on LPS-induced inflammation in male rats. Our results demonstrate that apigenin has anti-inflammatory effects in LPS treated rats, partially due to decreasing the IL-1β, IL-6, and TNF-α pro-inflammatory cytokine levels. This suppression effect has not a dose-dependent pattern. Our result showed that, compared to dexamethasone, the anti-inflammatory effect of apigenin is feeble, but 24 hours after induction of inflammation, apigenin has almost the the same anti-inflammatory effect as dexamethasone.

The injection of LPS, a component from the cell wall of gram-negative bacteria, is a relatively well-characterized model usage for activation of the immune system. LPS binds CD14 and Toll-like receptor 4 and can activate transcription factors, such as NF-κB. This transcription increases the production of pro-inflammatory cytokines (3).

IL-1β and IL-6 are a proinflammatory cytokine that regulates various physiological processes. Both play a vital role in the acute phase response and transition of acute inflammation to a chronic state. Evidence has accrued to suggest that dysregulation of IL-6 production is a major contributor to the pathogenesis of chronic inflammatory and autoimmune diseases. These Interleukins are made by white blood cells that interact with innate immunity and acquired immunity (15). Sepsis is one of the most critical and prevalent problems of infants, especially in low premature infants. Therefore, recognition of this disease is very important. Kocabaş *et al.* (2007) studied the role of IL-6 and TNF-α in the diagnosis of neonatal infections. The mean serum IL-6 and TNF-α levels were significantly higher in septal neonates before treatment than healthy neonates (16). Also, Boskabadi *et al.* (2013) evaluated the serum level of IL-6, 8, and 10 as the primary markers of infant infections. Their results showed a significant difference between the case and control groups at the level of IL-6, 8, and 10 ($P < 0.001$). IL-6 was the highest value for prediction of infection than other cytokines (17).

As an herbal compound, Flavonoids have beneficial effects on reducing inflammation and oxidative stress (13). Understanding the function and regulation of the prognostic factors involved in inflammation may provide new strategies for treating inflammatory diseases. Phytochemicals are natural ingredients with anti-inflammatory function which much attention is being paid to their benefits besides low toxicity and side effects. Recently, the use of herbal products to treat inflammatory diseases has increased as a new method, and the herbs medications have played a vital role in health care (18). The focus of drug discovery efforts has shifted from the lab bench to nature, and herbal medicines are still a rich source for the development of new drugs (19, 20). However, major barriers to the development of herbal medicines in the development of new drugs are the lack of scientific evidence of functional mechanisms, unknown toxicity, and drug interactions (21). Our findings in the present study provide important scientific evidence for the potential use of Apigenin as a therapeutic agent for inflammatory diseases. Therefore, our study focuses on the action of apigenin bioflavonoids in reducing pro-inflammatory cytokines. The apigenin is a beneficial molecule, mostly found in fruits and vegetables. The molecular and cellular effect of apigenin based on the recent studies origins through modulation of the gene expression of the cyclooxygenase, lipoxygenase, nitric oxide synthase, several inflammatory cytokines and the mainly

affects the mitogen-activated kinase (MAPK) and NF-κB and activator protein1 (AP-1) transcription factors that regulate many important biological and pathological processes (22). Due to their fundamental role in the intracellular signaling network and related transcription pathways, these factors have an appropriate target for therapy in inflammatory diseases (23). In this regard, Wang *et al.* (2015) showed that oxidative low-density lipoprotein (ox-LDL) maintains the survival of macrophages and induces secretion of protein-induced inflammatory cytokines to trigger in mice atherosclerosis. Prescription of apigenin can be reducing the number of foam cells and atherosclerosis, accompanying with reducing serum levels of TNF-α, IL-1β, IL-6. In fact, reducing macrophage-secretion of pro-inflammatory cytokines caused reduction of the inflammation and was followed by atherosclerosis (24). In this context, Weicheng *et al.* (2016) was studied the molecular mechanisms of inflammatory cytokines suppression of apigenin in RAW 264.7 macrophage cells, which stimulated by LPS. The researchers were reported that apigenin suppressed production of NO, PGE2, and TNF-α in RAW 264.7 in a dose-dependent manner. Additionally, apigenin inhibits the release of LPS-induced mRNA of iNOS, COX-2, and TNF-α. Treatment with apigenin reduced the transfer of c-Jun to the nucleus and reduced the activation of AP-1 by inhibiting MAPK and extracellular signal-regulated kinase (ERK) phosphorylation (25). Also, the protective effects of apigenin-enriched dietary were studied in a chronic colitis model induced by DSS in mice. The dietary apigenin supplementation in dietary decreased the damage signs of colitis, also reduced IL-1β and TNF-α proinflammatory cytokine secretions in primary LPS-stimulated solenocytes. Furthermore, the decreasing of IL-1β and IL-18 cytokine levels as a consequence of the regulation cleaved caspase-1 and caspase-11 enzymes (26, 27). Almost similar to our work, Karamese *et al.* (2016) investigated the protective effects of apigenin in a polymicrobial sepsis rat model. Based on their studies results, early treatment with apigenin significantly reduces the rate of inflammatory cells (28). The apigenin effects include inhibition of NF-κB pathway, suppression of inflammatory cytokines, and induction of anti-inflammatory cytokine production. According to evidence, inflammation may contribute to the pathophysiology of depression. So, its potential antidepressant activity was examined on LPS-induced depressive-like behavior in male mice. Pre-treatment with apigenin (25 and 50 mg/kg, IP) or fluoxetine (standard antidepressant drug) prevented LPS-induced abnormal behavior and attenuated production of IL-1β and TNF-α. Also, apigenin significantly reduced the expression of iNOS and COX-2 at both levels of mRNA and protein by modulating NF-κB in the prefrontal cortex (29, 30). This study evaluated the effect of pre-treatment with low doses of apigenin on the serums level of proinflammatory cytokine in LPS-stimulated rats. Our results were similar to the mentioned results of other studies. Further studies are needed to make decisions about choosing the most appropriate dosage, as well as for deciding how to administrate flavonoids to humans and obtaining authorization from the Food and Drug Administration of American (FDA). It needed to be given to allow for researchers to designed clinical trials and interventions, and in this way, there is a need to further studies to confirm or refute the use of this compound for humans.

Conclusion

Due to the effect of apigenin as a significant reduction in the level of inflammatory cytokines that is evaluated as a marker of suppression of inflammation, apigenin can be used for clinical trials for further medications.

Declarations

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Figures

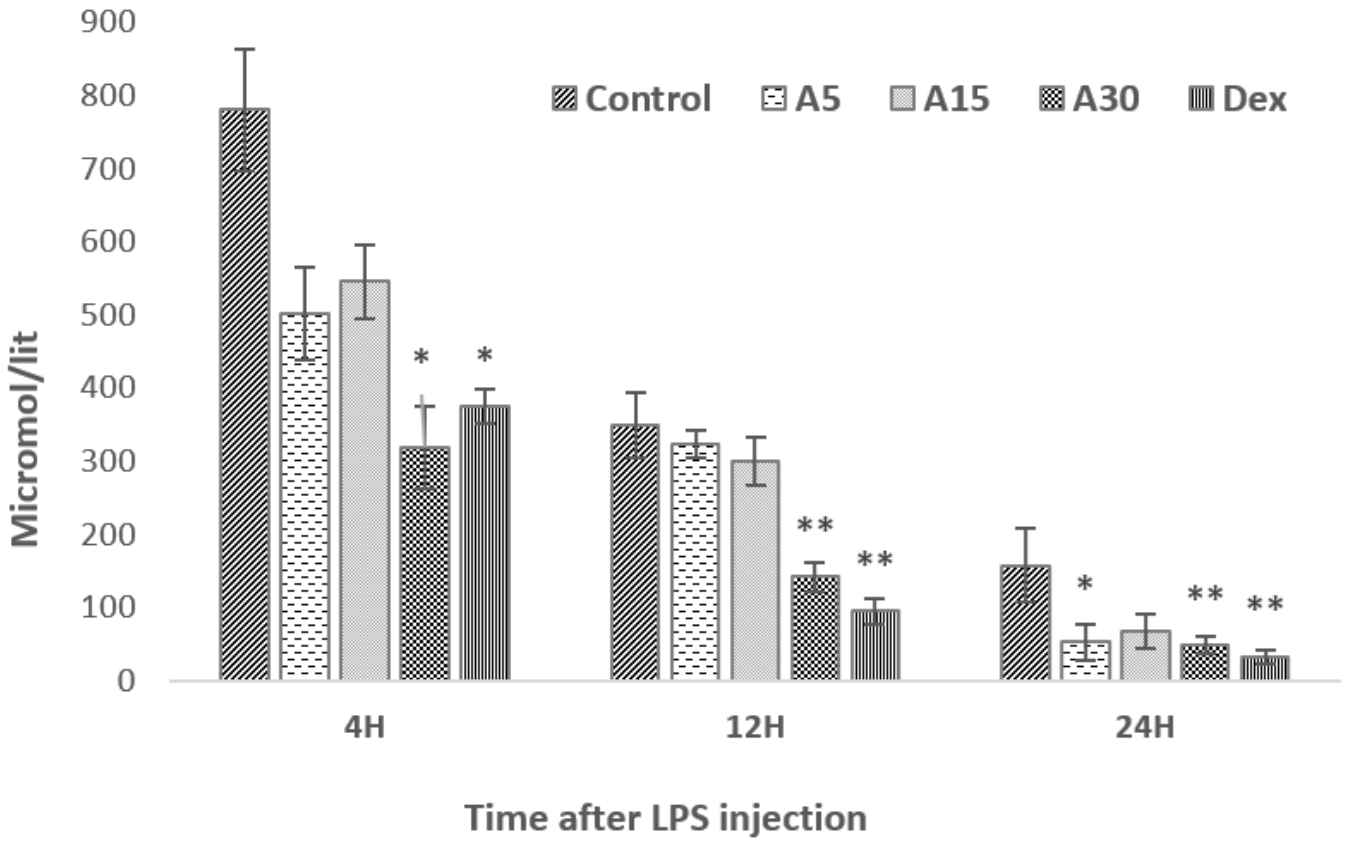

Figure 1

The TNF-α level at the times of intervals (4, 12, and 24 hours after LPS injection). IP pre-injection of 5 (A5), 15 (A15), and 30 (A30) mg/kg of apigenin and 15 mg/kg of dexamethasone (Dex) effect on TNF-α level. The results were normalized with a control group. (*p<0.05, **p<0.01)

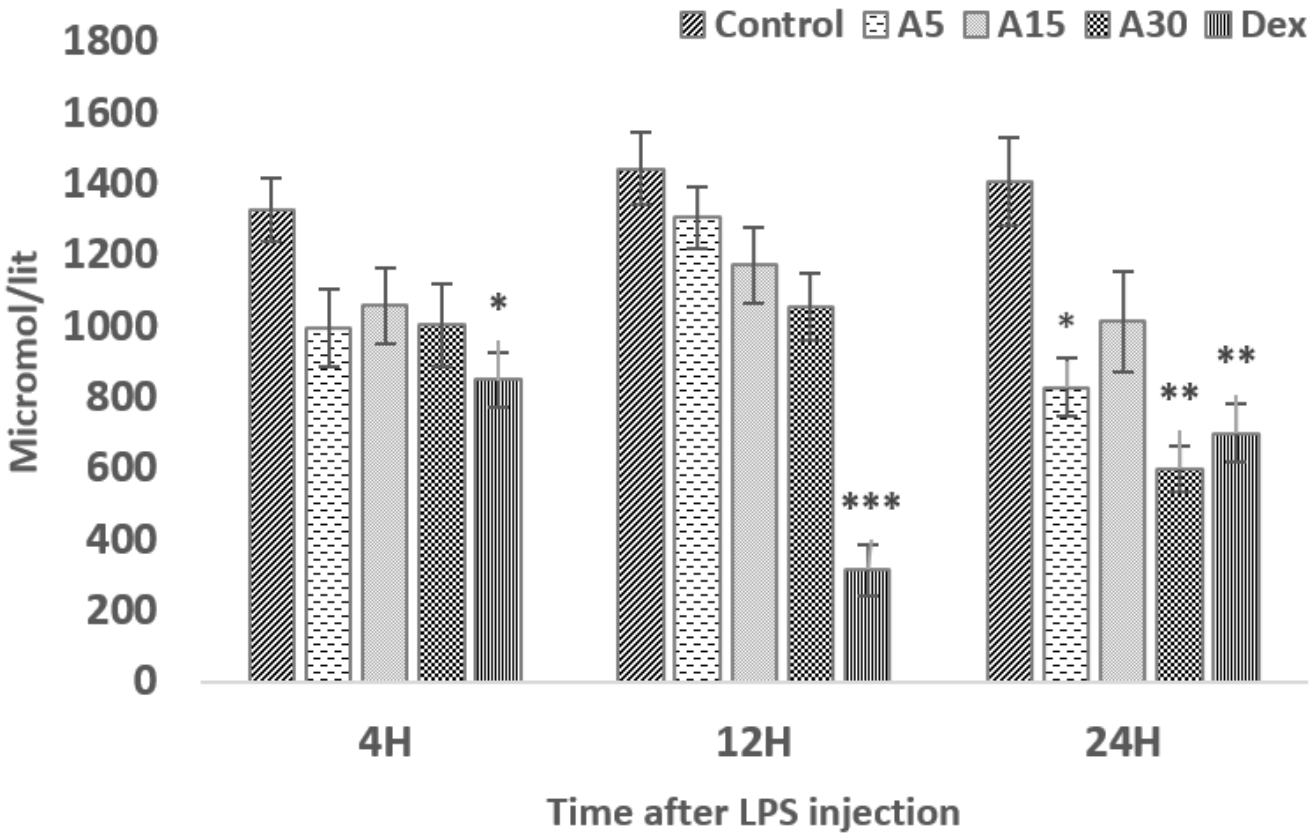

Figure 2

The IL-1β level at the times of intervals (4, 12, and 24 hours after LPS injection). IP pre-injection of 5 (A5), 15 (A15) and 30 (A30) mg/kg of apigenin and 15 mg/kg of dexamethasone (Dex) effect on IL-1β level. The results were normalized with a control group. (*p<0.05, **p<0.01, ***p<0.001)

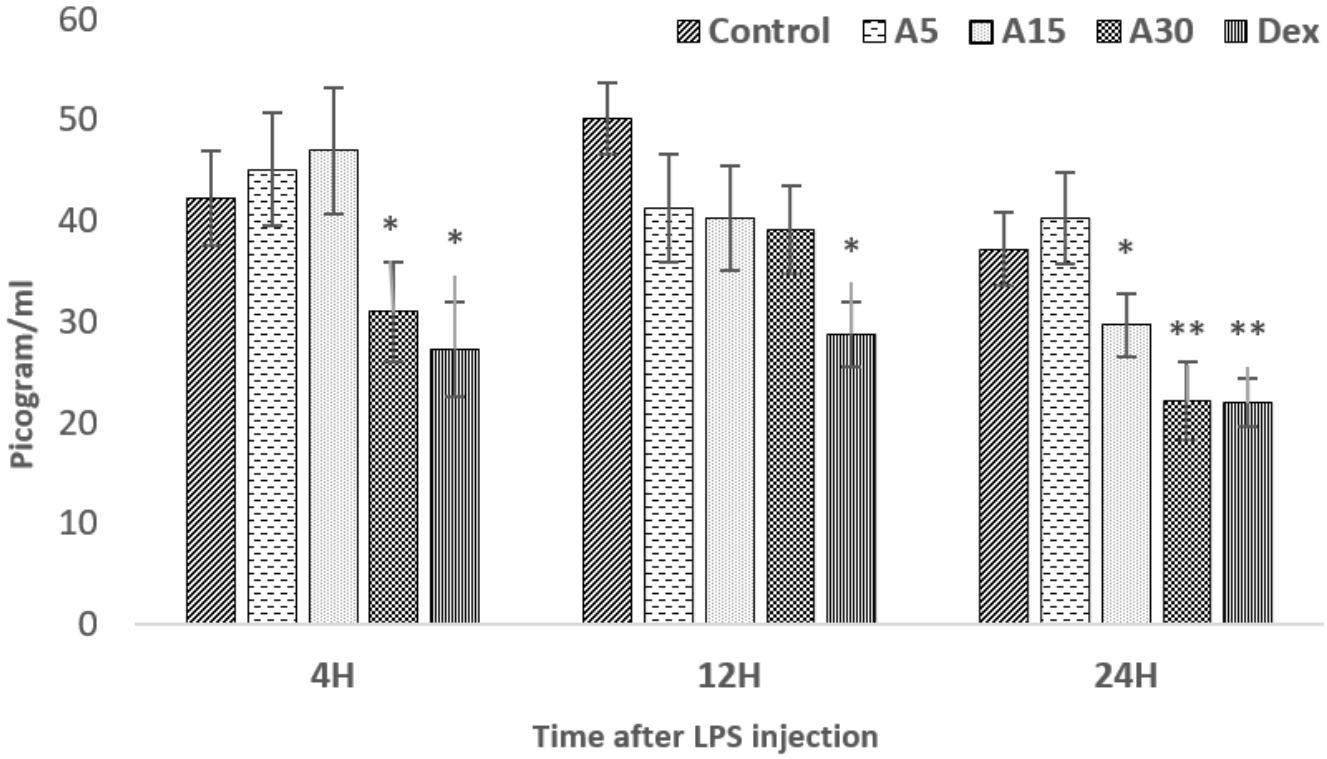

Figure 3

IL-6 level at the times of intervals (4, 12, and 24 hours after LPS injection). IP pre-injection of 5 (A5), 15 (A15), and 30 (A30) mg/kg of apigenin and 15 mg/kg of dexamethasone (Dex) effect on IL-6 level. The results were normalized with a control group. (*p<0.05, **p<0.01)