CHAPTER IV

RESULTS

4.1 Mitogenic of *S. scaphigerum* fruit extract on human PBMCs

Human peripheral blood mononuclear cells were treated with various concentrations of *S. scaphigerum* and PHA. The RPMI 1640 medium and PHA were used as the negative and positive controls, respectively.

The suitable concentration of PHA for using as a positive control was first evaluated by MTT assay. The result in Figure 4.1 showed that PHA could significantly stimulate the cells in concentration dependent manner. The percentage of stimulating effect of PHA was 21.84 ± 3.81, 53.08 ± 8.56, 102.32 ± 10.61 and 85.37 ± 4.3 at the concentrations of 5, 10, 50 and 100 μg/ml, respectively. The concentration, which produced 50% stimulation, was used as the positive control for further studies.

The *S. scaphigerum* fruit extract, at 10 to 500 μg/ml, could stimulate human PBMCs in a concentration dependent manner. The percentage of stimulation was -20.12 ± 4.96, -6.92 ± 4.15, 19.89 ± 2.78, 105.53 ± 7.07 and 169.10 ± 9.25 at concentrations of 10, 30, 100, 300 and 500 μg/ml, respectively while 10 μg/ml PHA stimulated the cells at 71.42 ± 4.30 %. The 50% stimulatory concentration of the extract was 150 μg/ml (Figure 4.2). This concentration was selected in the next experiment.

The mitogenic activity of the extract was confirmed by Tritiated thymidine incorporation assay. The result demonstrated that the extract significantly stimulated human PBMCs proliferation in a concentration dependent pattern (Figure 4.3). The percentage of stimulating effect of the extract was 32.34 ± 3.92, 69.01 ± 5.64 and 145.86 ± 10.78 at concentrations of 75, 150 and 300 μg/ml, respectively. However, it

had much less potent mitogenic effect when compared with to 10 μg/ml PHA which stimulated cells at 23,687.74 ± 4,690.54%.

4.2 Effect of *S. scaphigerum* fruit extract on T cell activation

This effect of the extract was evaluated by determining CD69 expression on $CD3^+$ T cells by using FACS. CD69 is the marker molecule on T lymphocyte which is expressed only at the early state of T cell activation.

The result in Figure 4.4 demonstrated that the extract could slightly increase the percentage of CD69 expression on $CD3^+$ T cells to 0.66 ± 0.16, 0.80 ± 0.17, 1.00 ± 0.13 and 1.45 ± 0.07 at concentrations of 75, 150, 300 and 600 μg/ml, respectively. This increase was not significant when compared to the basal level (0.44±0.14) of expression in the untreated condition. The positive control, 10 μg/ml PHA significantly stimulated the CD69 expression (25.33 ± 3.91 %) (Figure 4.5).

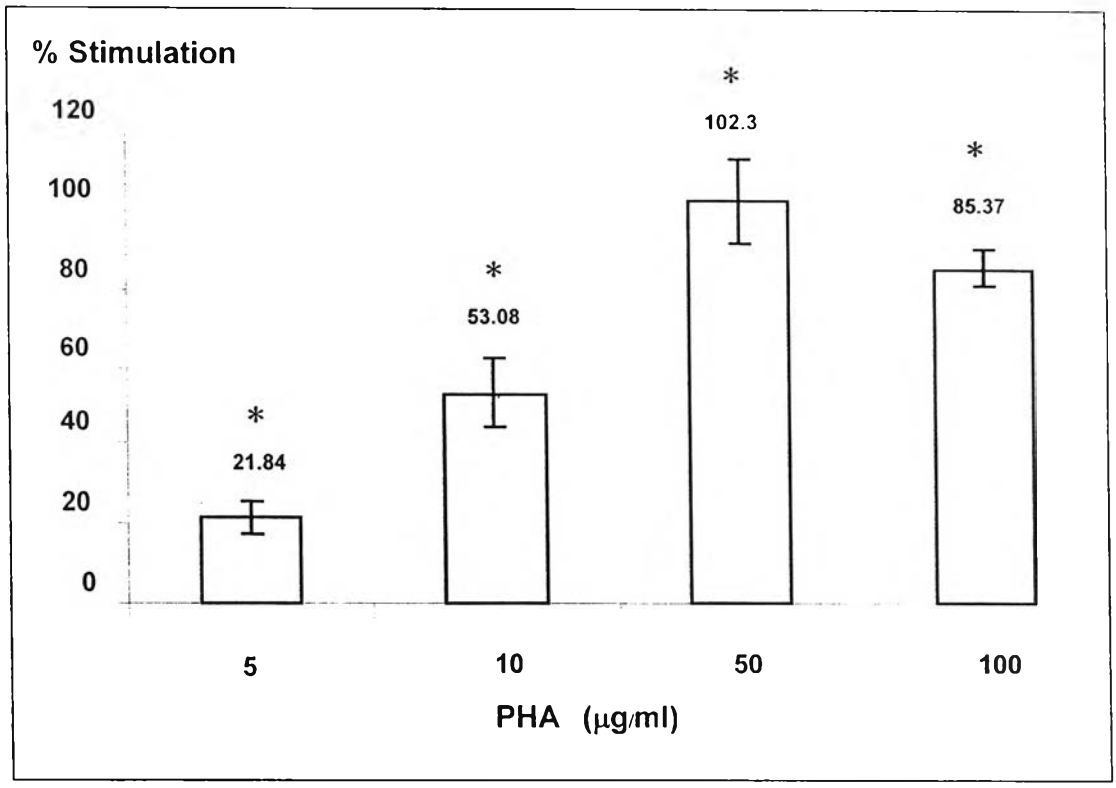


Figure 4.1 Mitogenic activity of PHA on human PBMCs. Cells were treated with various concentrations of PHA for 48 hours and mitogenic activity was performed using MTT assay. The results were expressed as the percentage of stimulation over untreated control (mean± S.E.M.), (n=3). * $p < 0.05$ compared with the untreated control.

Figure 4.2 Immunostimulation of *S. Scaphigerum* fruit extract on human PBMCs. Cells were treated with various concentration of the extract for 48 hours and the stimulatory effect was determined by MTT assay. The percentage of stimulation over the untreated control was plotted against the concentrations of the extract. The results were expressed as mean± S.E.M. (n=5). * $p < 0.05$ compared with the untreated control. As positive control, 10 µg/ml PHA stimulation was 71.42 ± 4.30%.

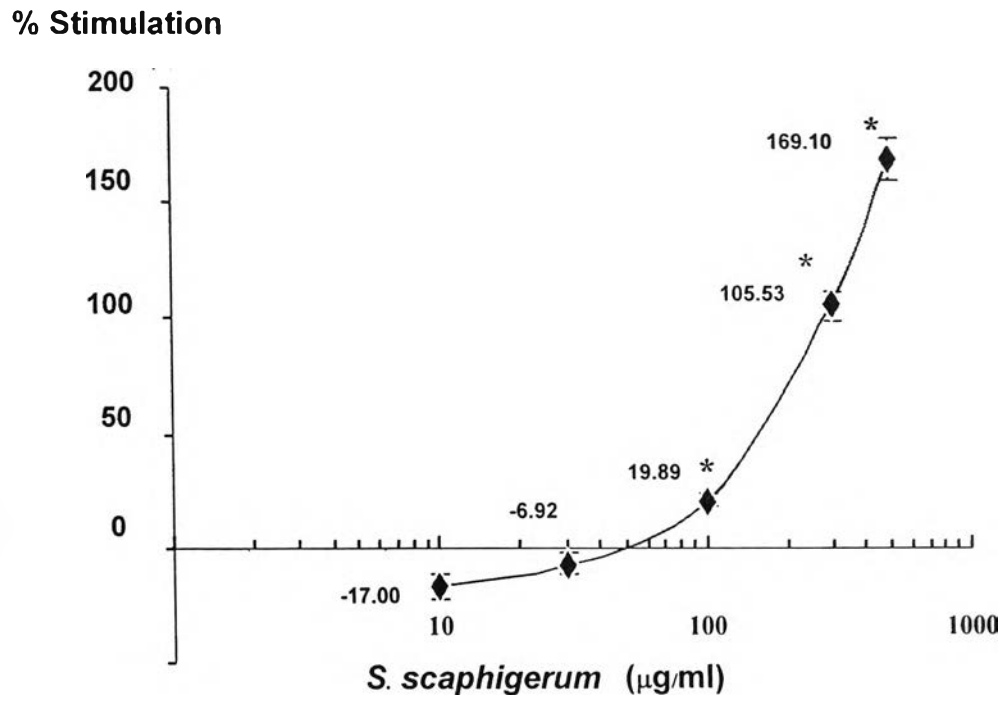


Figure 4.3 Effect of *S. scaphigerum* fruit extract on human PBMCs proliferation. Cells were treated with the extract (0-300 μg/ml) for 48 hours and cell proliferation was evaluated by ^{3}H-thymidine incorporation assay. The results were represented as the percentage of stimulation over the untreated control (mean± S.E.M., n=5). * $p < 0.05$) compared with untreated control. As positive control, 10 μg/ml PHA stimulation was 23,687 ± 4,690.54 %.

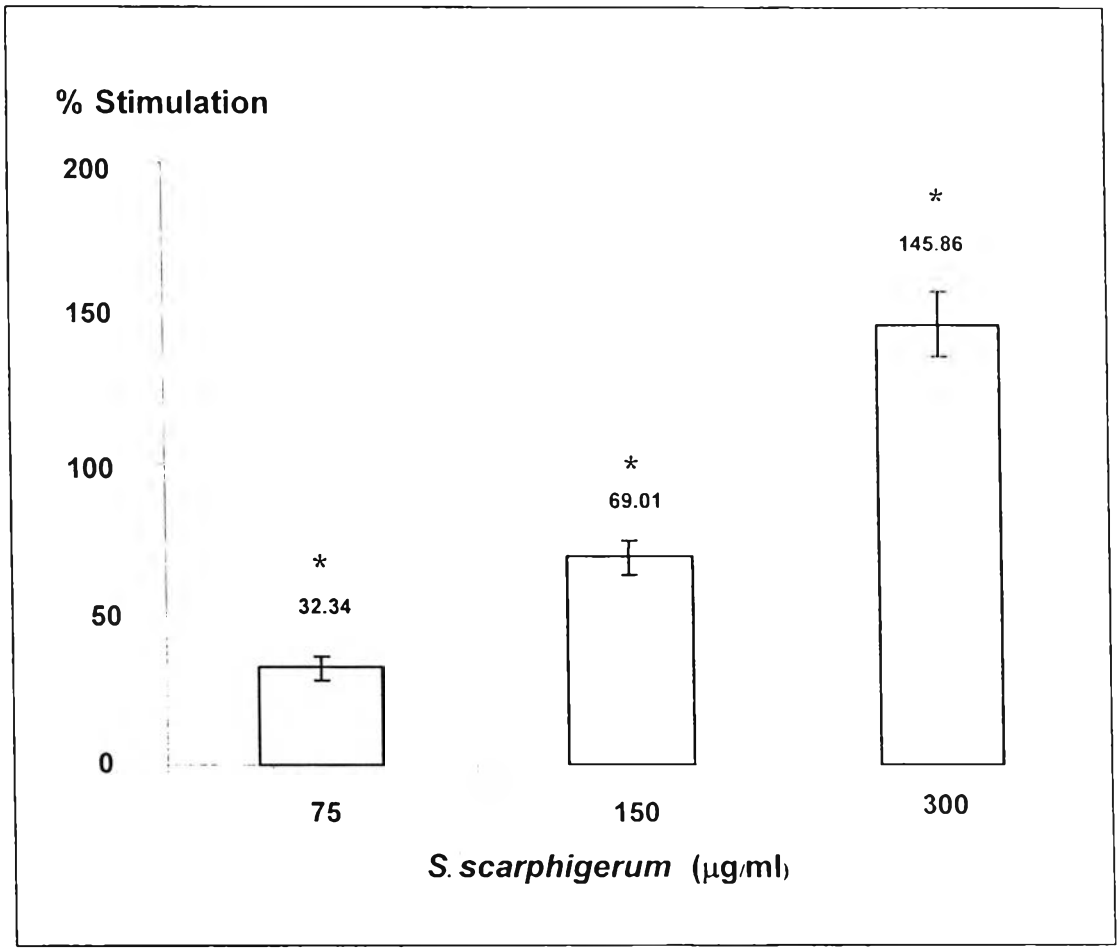


Figure 4.4 Effect of *S. scaphigerum* fruit extract on CD69 expression on CD_3^+ T cells. Human PBMCs were treated with the extract (0-600 μg/ml) for 24 hours. The cells were stained with CD3-perCP and CD69-PE and analysed by flow cytometer. The result were represented as the percentage of CD3/CD69 expression (n=5). As positive control, 10 μg/ml PHA stimulation was 25.33 ± 3.91 %.

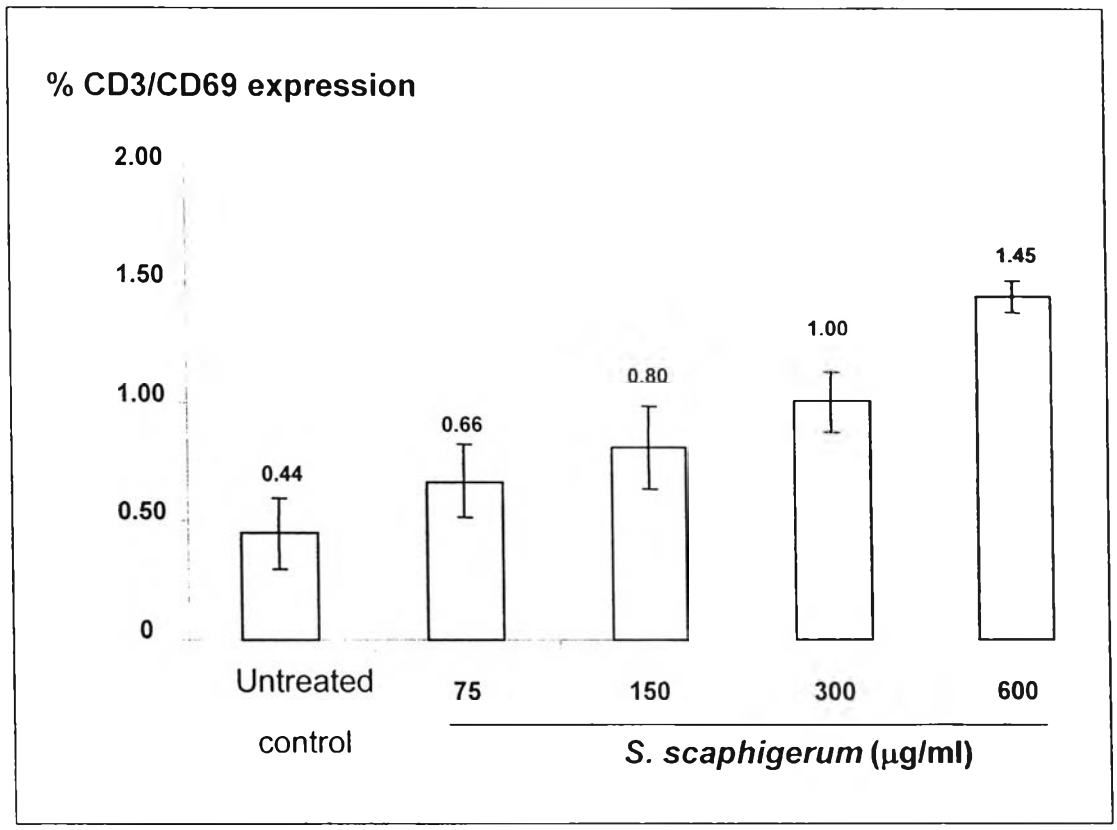
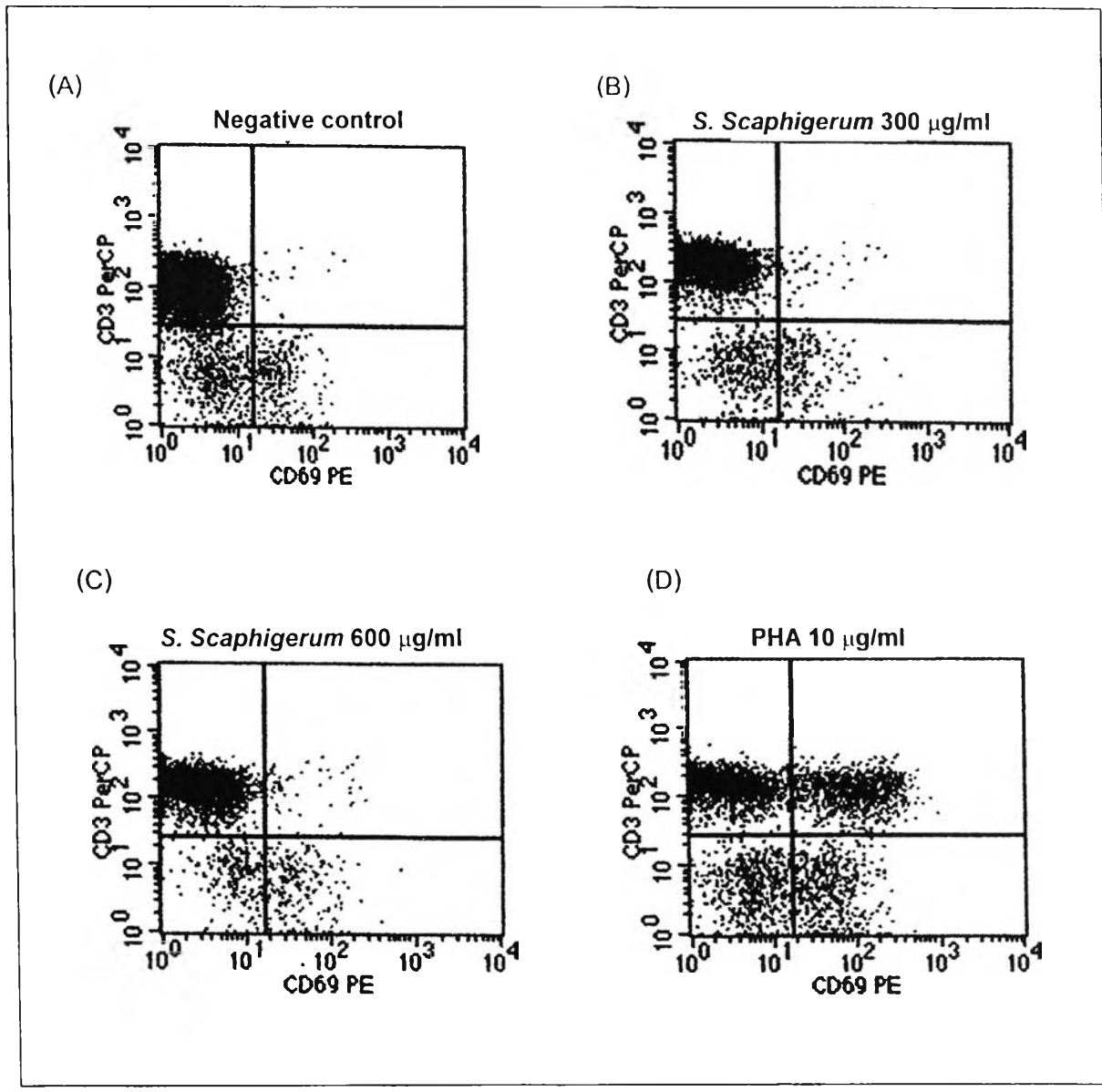


Figure 4.5 Representative fluorescence cytograms demonstrating CD69 expression by gated CD3 cells. (A) treated with RPMI 1640 medium as negative control; (B),(C) treated with 300 and 600 μg/ml *S. scaphigerum* fruit extract; (D) treated with 10 μg/ml PHA. The number in the upper right quadrant of each panel represents the percentage of CD3 cells expressing CD69.



4.3 Effect of *S. scaphigerum* fruit extract on phagocytosis of J774A.1 cells

Phagocytosis of zymasan and nitroblue tetrazolium (NBT) by macrophage J774A.1 cells was used for determining the effect of *S. scaphigerum* fruit extract. Lipopolysaccharide (LPS) was used as the positive control. *S. scaphigerum* fruit extract stimulated phagocytosis activity of J774A.1 cells in a concentration dependent manner when compared with the non-stimulated condition (Figure 4.6).

The result in Figure 4.6 demonstrated that the percentage of stimulation of the extract was 8.62 ± 0.16, 21.50 ± 3.75, 33.68 ± 3.13 and 43.87 ± 2.56 at concentrations of 75, 150, 300 and 600 μg/ml, respectively while 5 μg/ml LPS stimulation was 33.44 ± 1.79 %.

4.4 Effect of *S. scaphigerum* fruit extract on nitric oxide production of J774A.1 cells

One parameter commonly used as a marker for macrophage activation is nitric oxide (NO). Due to the lability of NO, the stable end products, nitrate and/or nitrite were used as markers of this labile agent. These end products are usually determined by Griess reaction.

The suitable concentration of LPS, the positive control, was evaluated before use. The result in Figure 4.7 demonstrated that LPS activated the NO production in concentration dependent pattern. The amount of nitrite was 8.56 ± 0.78, 9.58 ± 0.82, 10.60 ± 0.63 and 12.81 ± 1.06 at concentrations of 1, 2.5, 5 and 10 μg/ml, respectively.

S. scaphigerum fruit extract significantly stimulated NO production from J774A.1 cells at the concentration dependent pattern. The amount of nitrite was 7.47 ± 0.21, 8.56 ± 0.25, 9.60 ± 0.38 and 15.01 ± 0.89 at concentrations of 75, 150, 300 and 600 μg/ml, respectively while 1 μg/ml LPS increased the NO production of the cells up to 12.06 ± 0.25 % (Figure 4.8).

Figure 4.6 Effect of *S. scaphigerum* fruit extract on phagocytotic activity of J774A.1 cells. Cells were treated with 0-600 μg/ml of the extract for 24 hours and the stimulatory effect was determined by phagocytosis of zyrmasan and NBT assay. The percentage of stimulation over the untreated control was plotted against the concentrations of the extract. The results were expressed as mean± S.E.M. (n=5). * $p < 0.05$ compared with the untreated control.

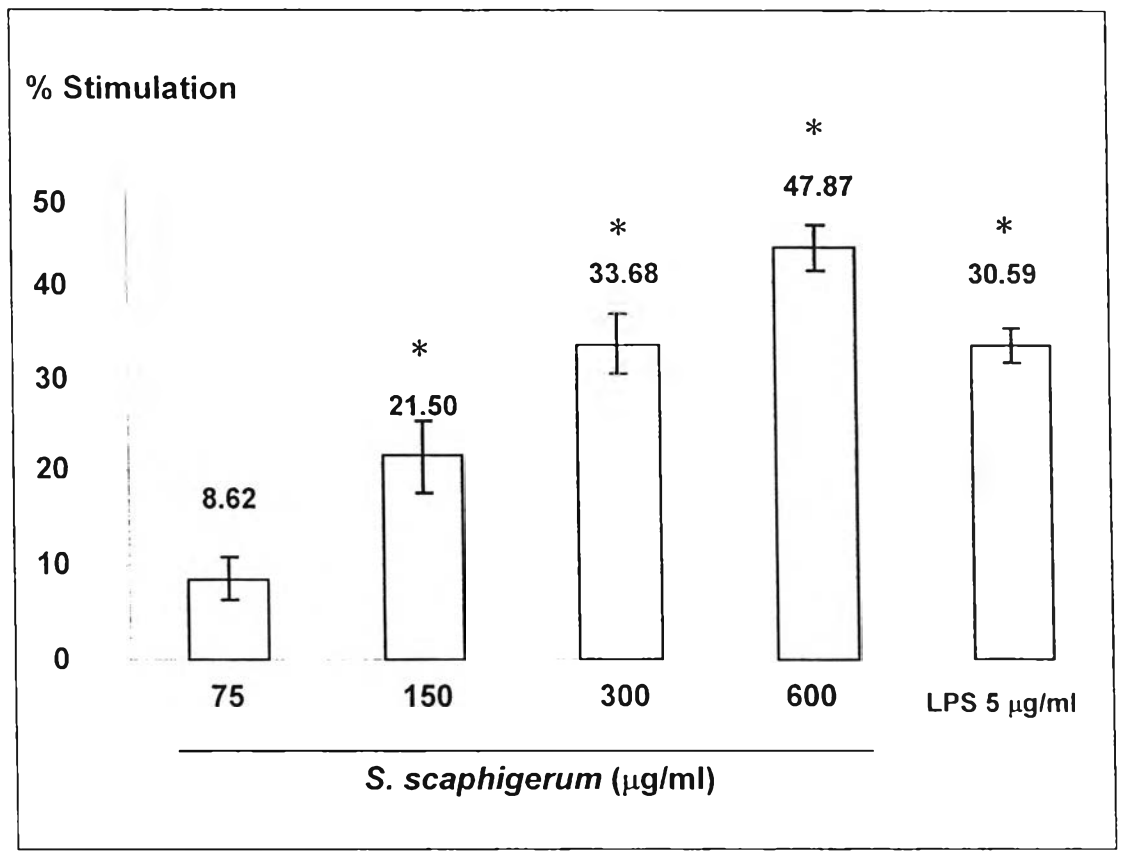


Figure 4.7 Effect of LPS on nitric oxide release from J774A.1 cells. Cells were treated with 0-10 μg/ml of LPS for 24 hours and nitrite (NO_2) was evaluated by Griess reaction assay. The results were represented as the amount of nitrite (μM) (mean± S.E.M., n=2). * $p < 0.05$) compared with untreated control.

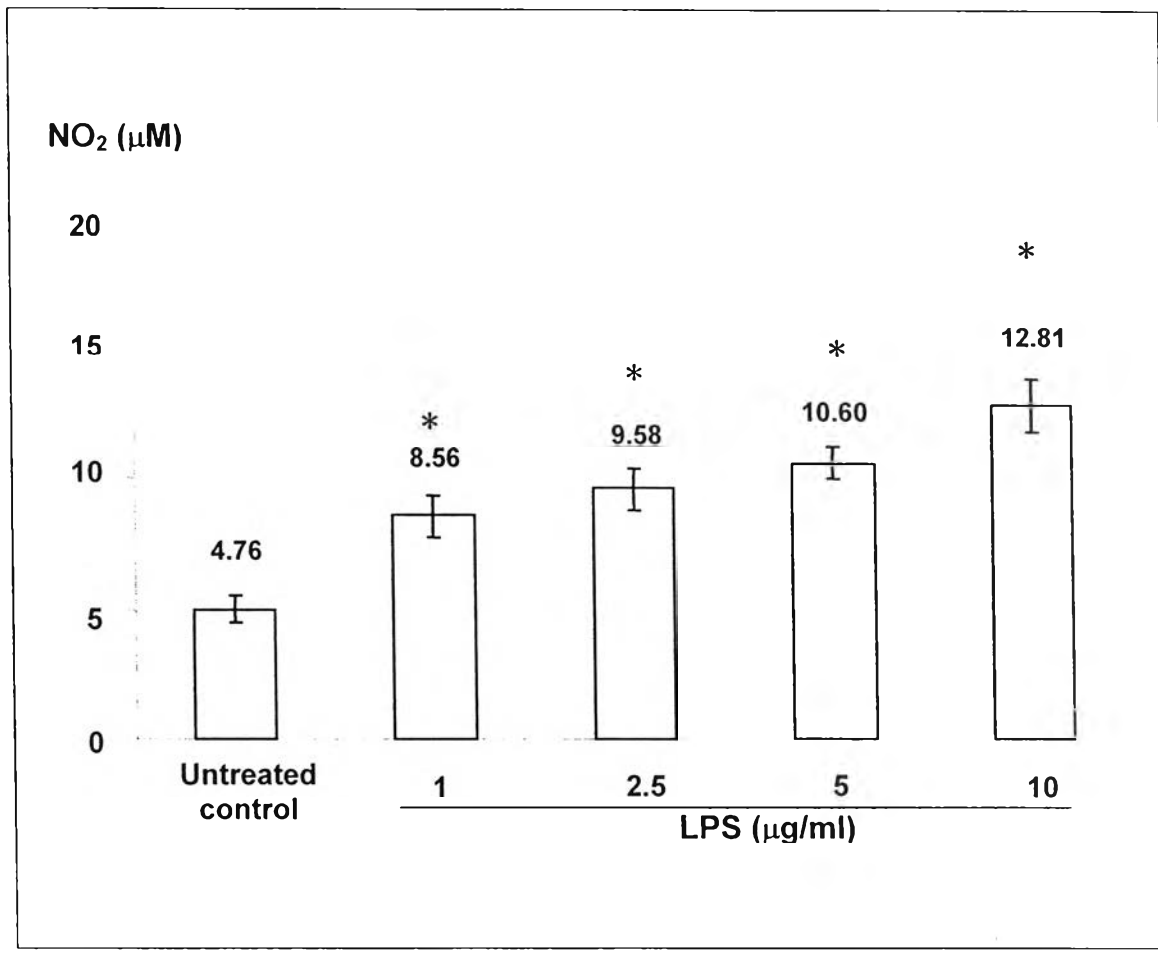


Figure 4.8 Effect of *S. scaphigerum* fruit extract on nitric oxide release from J774A.1 cells. Cells were treated with 0-600 μg/ml of the extract for 24 hours and nitrite (NO_2) was evaluated by Griess reaction assay. The results were presented as the amount of nitrite (μm) (mean± S.E.M., n=3). * p <0.05 compared with untreated control.