

# ***In vitro* Immuno-modulation Effect of Polysaccharides Fractions from American Ginseng Root**

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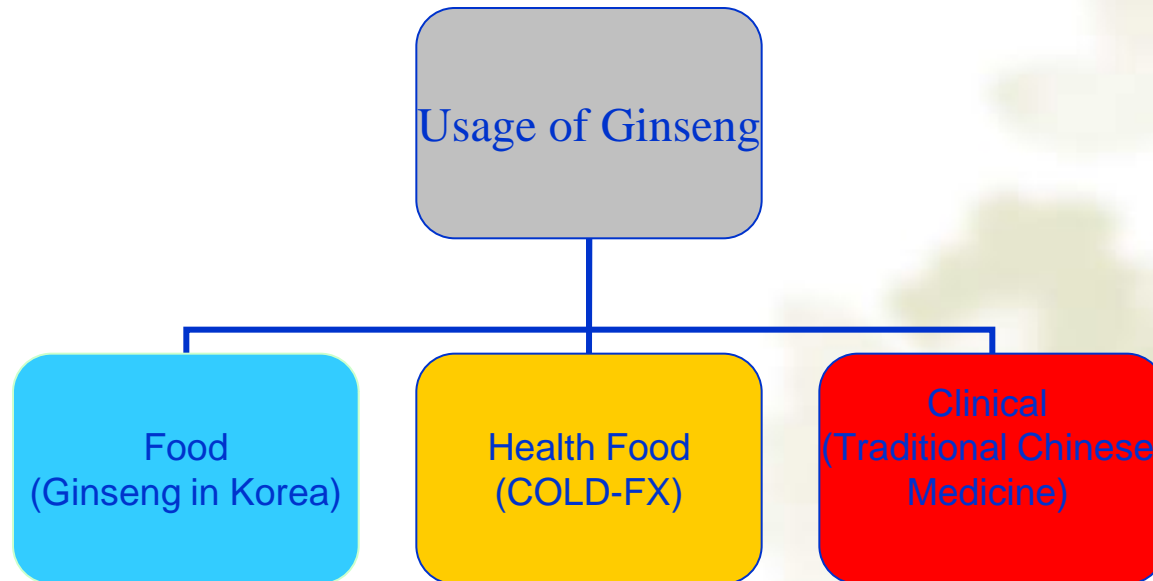
Supervisor: Dr. Lui and Prof. Ren

# Acknowledgement

- ❖ (1) Prof. Ren who supervised the project and provided me the opportunity to come here.
- ❖ (2) Dr. Lui who invite me to come and aided in the Pharmacological experimental design.
- ❖ (3) Miss Pei for her guidance in conducting my experiment.

# 1. Background

- American ginseng (*Panax quinquefolius L.*) is one of the most recognized ginseng botanicals around the world.
- Used as food, health food and drug for the pharmacological effects on the central nervous system, cardiovascular system, endocrine system, immune system and cancer.



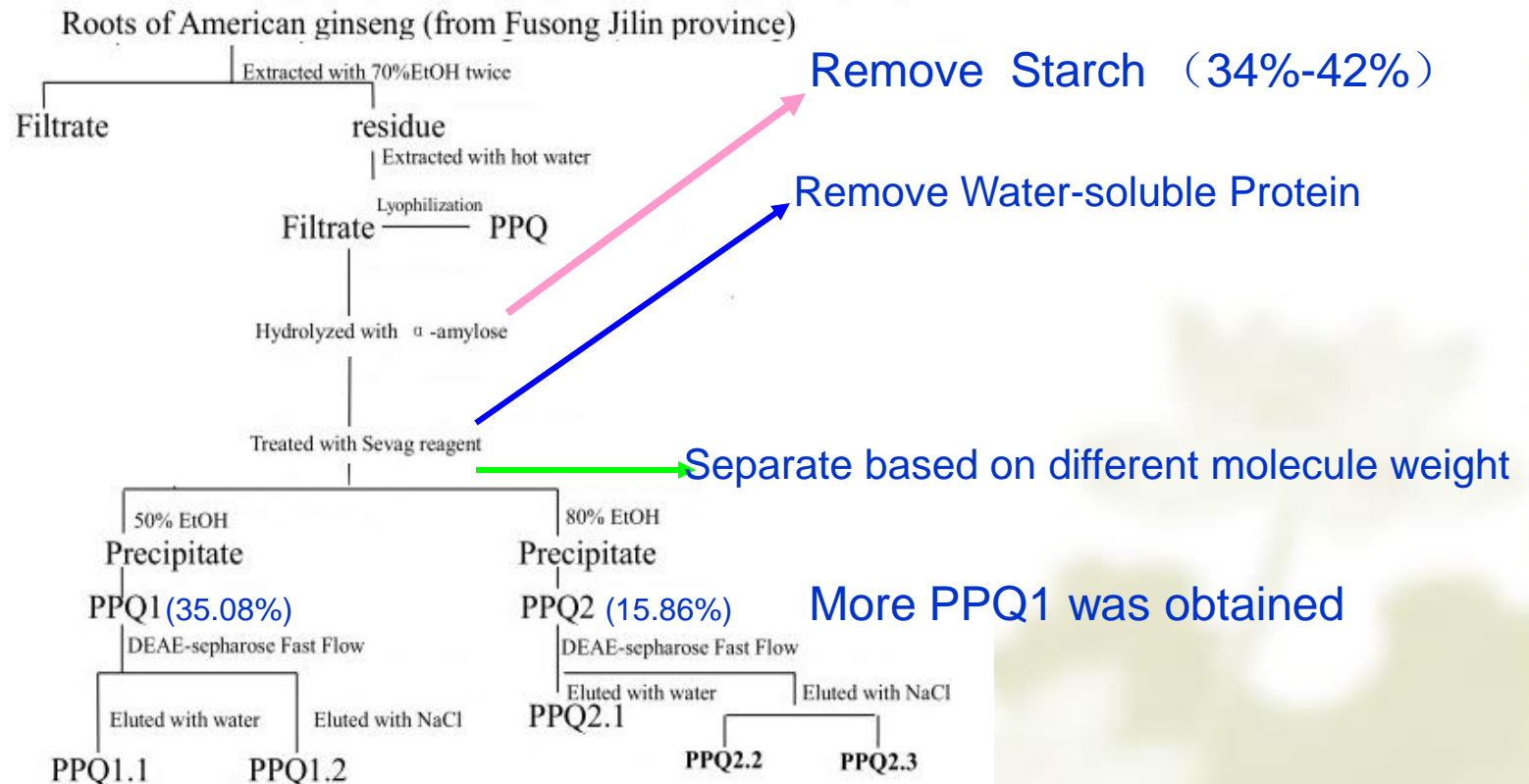
- ❖ Polysaccharides,
- 5-10%,
- ❖ gain more and more interest for it's
- immuno-modulatory ( innate and adaptive)
- anti-tumor, anti-adhesive,
- antioxidant,
- hypoglycemic effects.

## 2.Objectives of my study

- ❖ (1) Extraction, purification and fractionation polysaccharides from American ginseng root
  - Chemical structure identification:
  - molecular weight,
  - monosaccharides,
  - spatial structure feature (NMR ).
- ❖ (2) Assay the immuno-modulatory effects of polysaccharide fractions on macrophages.
  - Immuno-stimulation
  - Anti-inflammation.

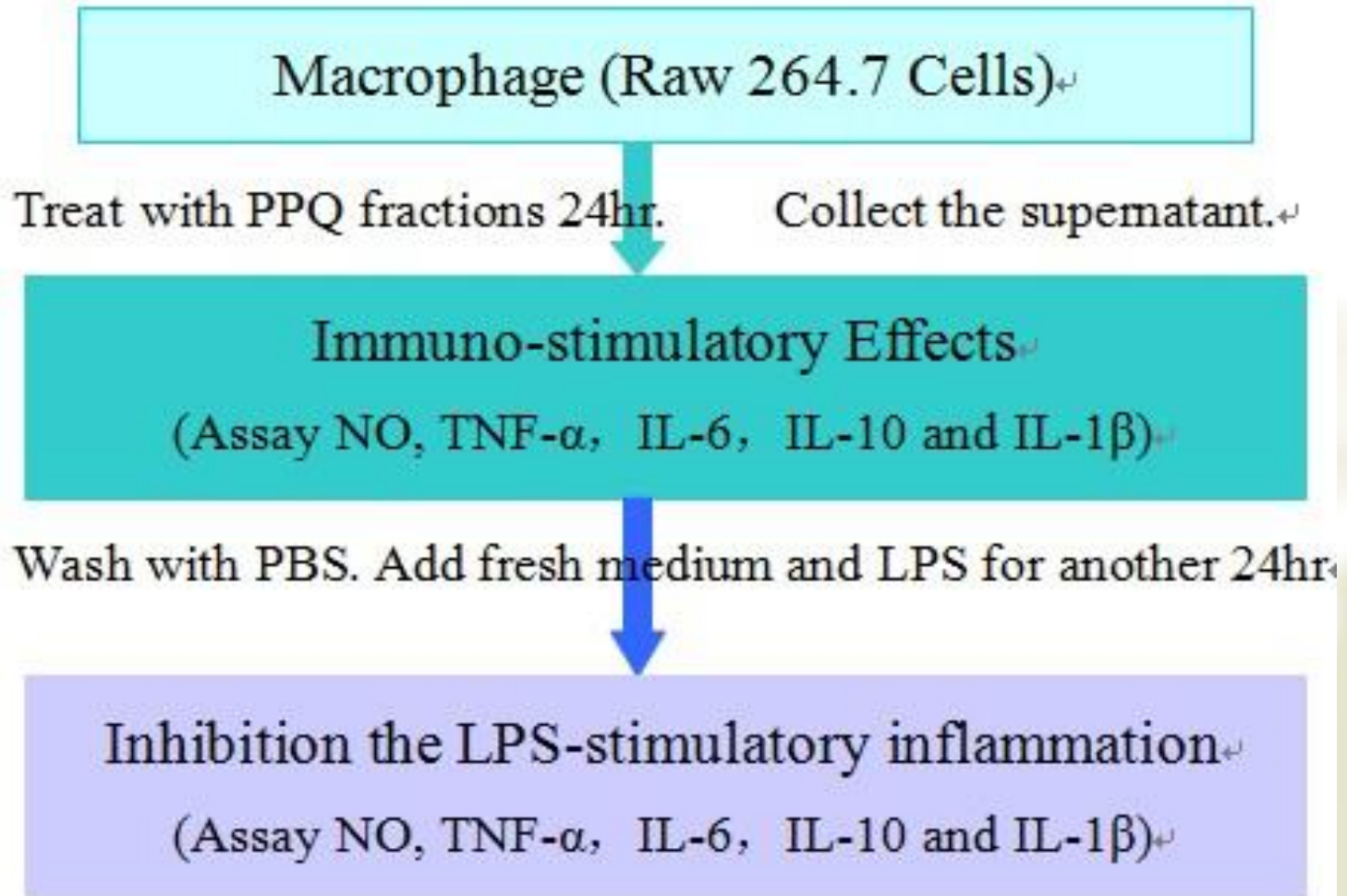
# 3. Experiment Design

## 3.1. Extraction, Purification and Fractionation



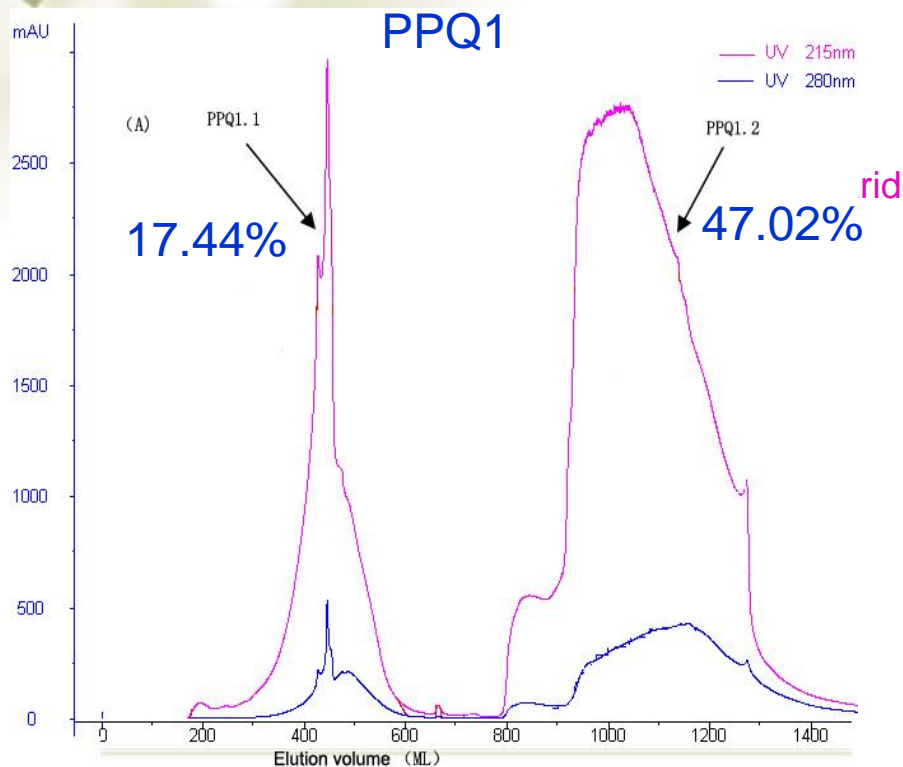
49% of the compositions were removed from PPQ with  $\alpha$ -amylase and Sevag-reagent

## ❖ 3.2 Pharmacological Characterization

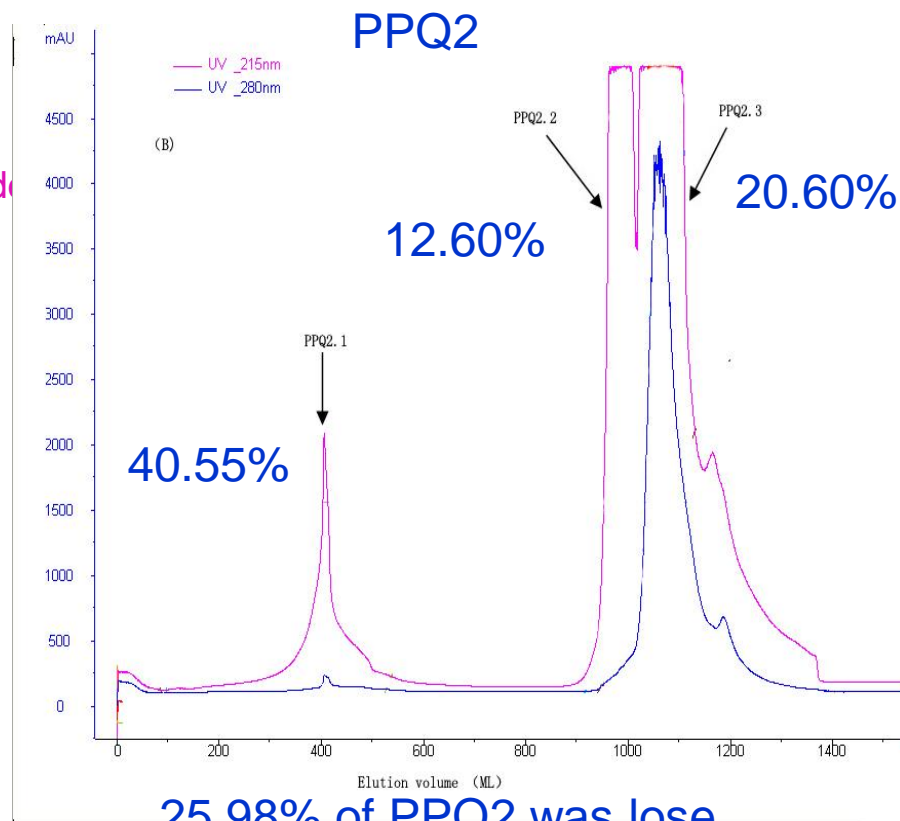


# 4. Result and Discussion

## 4.1 Anion Exchange Chromatography



35.23% of PPQ1 was lose



25.98% of PPQ2 was lose

PPQ2 had higher total yield ratio (74.02%) than PPQ1 (64.77%). More acidic fraction (47.33%) in PPQ1

PPQ2.3 Maybe a glycoprotein for the high absorbance at 280 nm

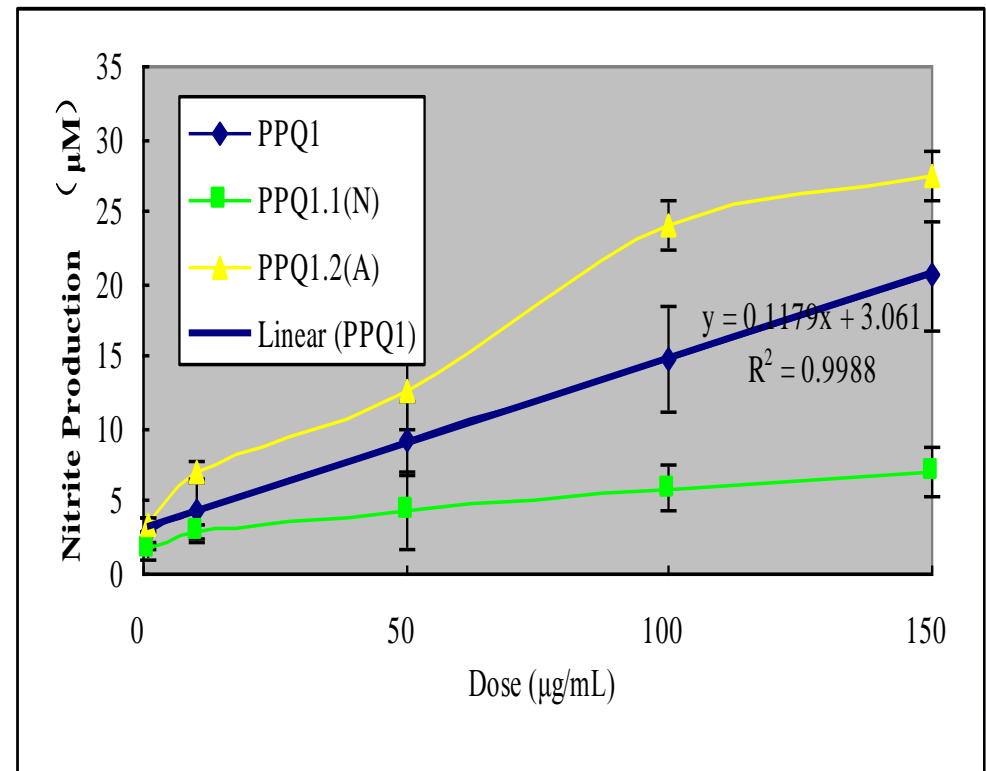


# 4. Result and Discussion

## 4.2 In vitro immuno-stimulatory effect

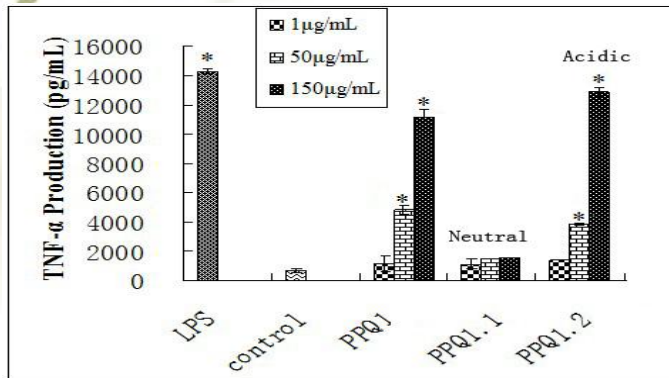
### 4.2.1 Effect on the NO production

- PPQ1 was effective and the Dose-Response curve was linear with  $R^2=0.9988$ .
- Acidic fraction (PPQ1.2) was more effective than PPQ1 and neutral fraction (PPQ1.1) less effective.
- A similar result was got from PPQ2 and its sub-fractions.

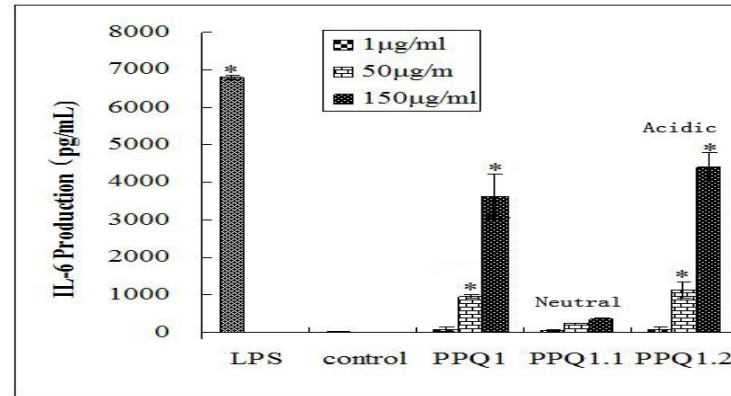


## 4. 2.2 Effects of PPQ1 and it's sub-fractions on the cytokine production

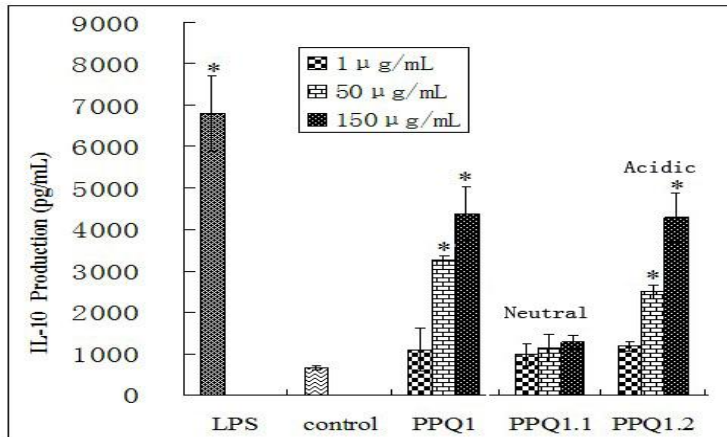
(a) TNF- $\alpha$



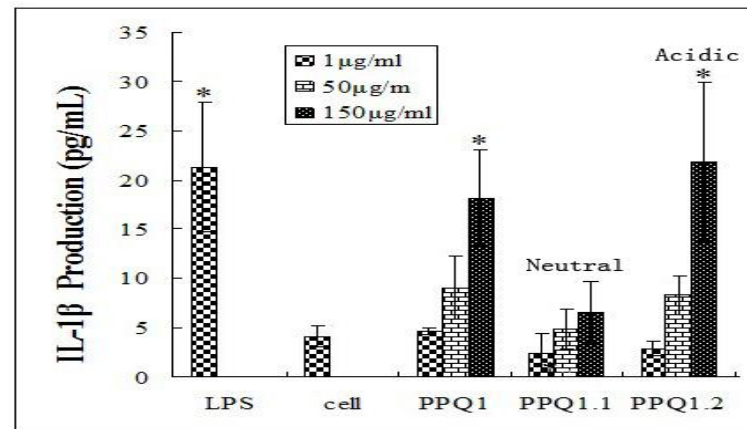
(b) IL-6



(c) IL-10



(d) IL-1 $\beta$



PPQ1 and it's sub-fractions had similar effects on this four cytokines (TNF- $\alpha$  ,IL-6, IL-10 and IL-1 $\beta$ ). Neutral fraction (PPQ1.1) was inactive. Crude fraction and acidic fraction up-regulated the production and acted in a concentration-dependent manner. There was no obvious difference between PPQ1 and acidic fraction PPQ1.2. IL-1 $\beta$ production was very low compared with TNF- $\alpha$  ,IL-6, IL-10

# 4.3 Inhibition on LPS-stimulatory inflammation effect

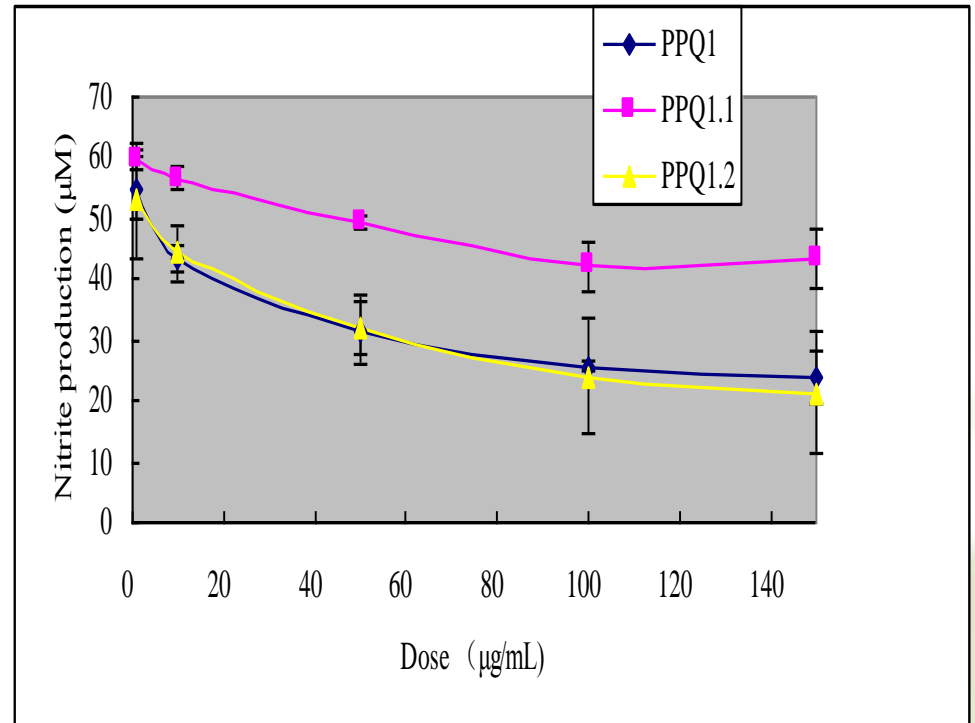
## 4.3.1 Down-regulation on the NO production

Not very obvious difference between PPQ1 and its acidic fraction PPQ1.2.

PPQ1 not acted in the linear way.

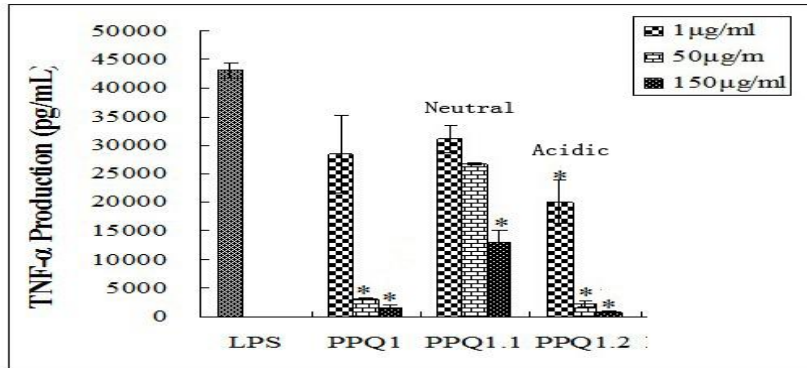
Although no effect on the immuno-stimulatory effect PPQ1.1 showed inhibition on the LPS-stimulatory NO production.

Nitrite production

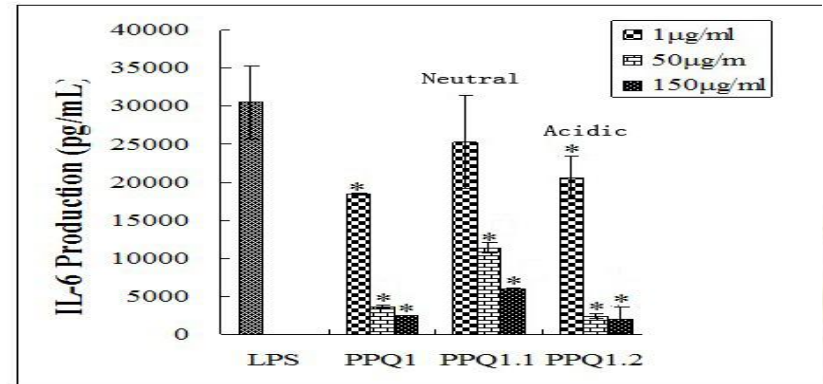


### 4.3.2 Down-regulation on cytokines production

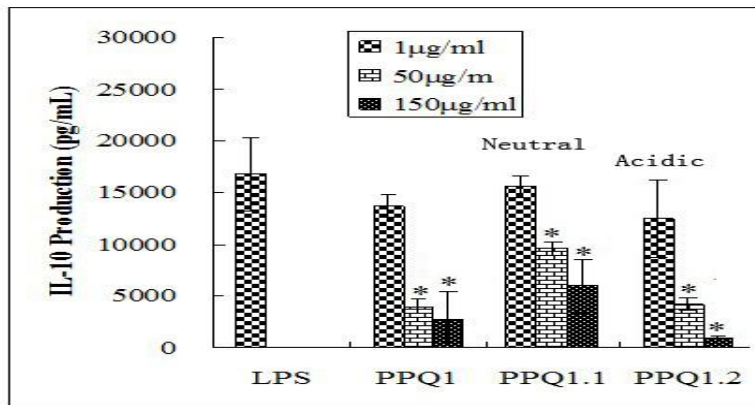
(a) TNF- $\alpha$



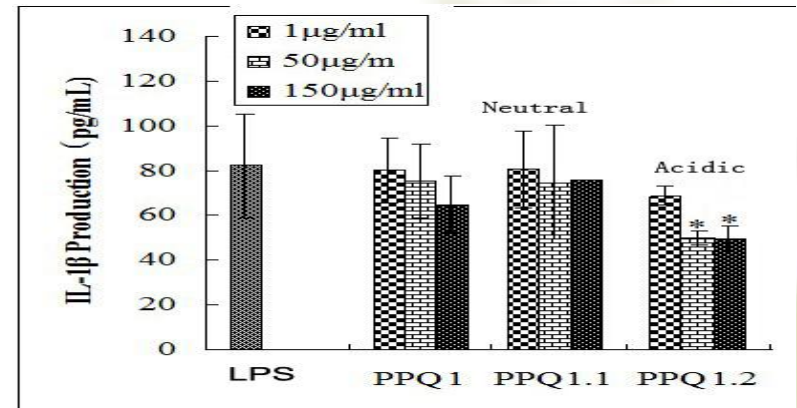
(b) IL-6



(c) IL-10



(d) IL-1 $\beta$



Neutral fraction showed inhibition effects on the cytokine (TNF- $\alpha$ , IL-6 and IL-10) production but weaker than crude fractions and acidic fractions.

The cytokine (TNF- $\alpha$ , IL-6, IL-10) production dropped rapidly when the treatment concentration increased from 1  $\mu\text{g/ml}$  to 50  $\mu\text{g/ml}$ .

# 5. Conclusion

- ❖ 1. DEAE-sepharose Fast Flow is effective in the fractionation.
- ❖ 2. More acidic fractions than neutral fractions in the AN ginseng root (1.75:1).
- ❖ 3. Acidic fractions were more active in immuo-modulation effects of macrophages than neutral fractions.
- ❖ 4. Although neutral fractions were inactive in the immuno-stimulation they still useful for displayed the inhibition against LPS-stimulatory inflammation.
- ❖ 5. Immuno-modulation activity of polysaccharides were affected by;
  - ❖ Molecule weight,
  - ❖ State of purity,
  - ❖ Charge



***Thanks for Your  
attendance!***

