



兽医学（中医药）北京市重点实验室
Beijing Key Lab of Traditional Chinese Veterinary Medicine
Beijing University of Agriculture



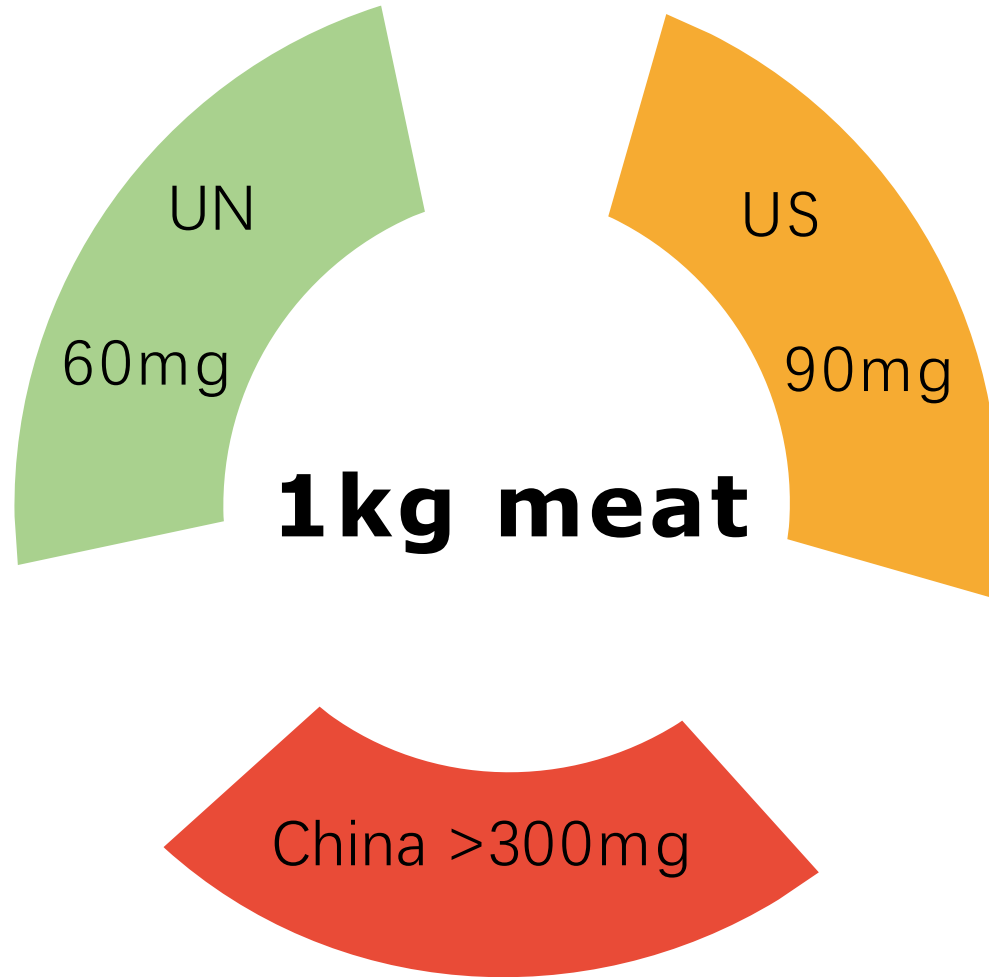
Mechanisms of Pulsatilla Decoction in the Treatment of Diarrhea Caused by *Escherichia coli*

Hong Dong Professor

**3rd International Symposium on
Alternatives to Antibiotics (ATA)**

*The Berkeley Hotel, Bangkok, Thailand,
December 16-18, 2019*

Antibiotics





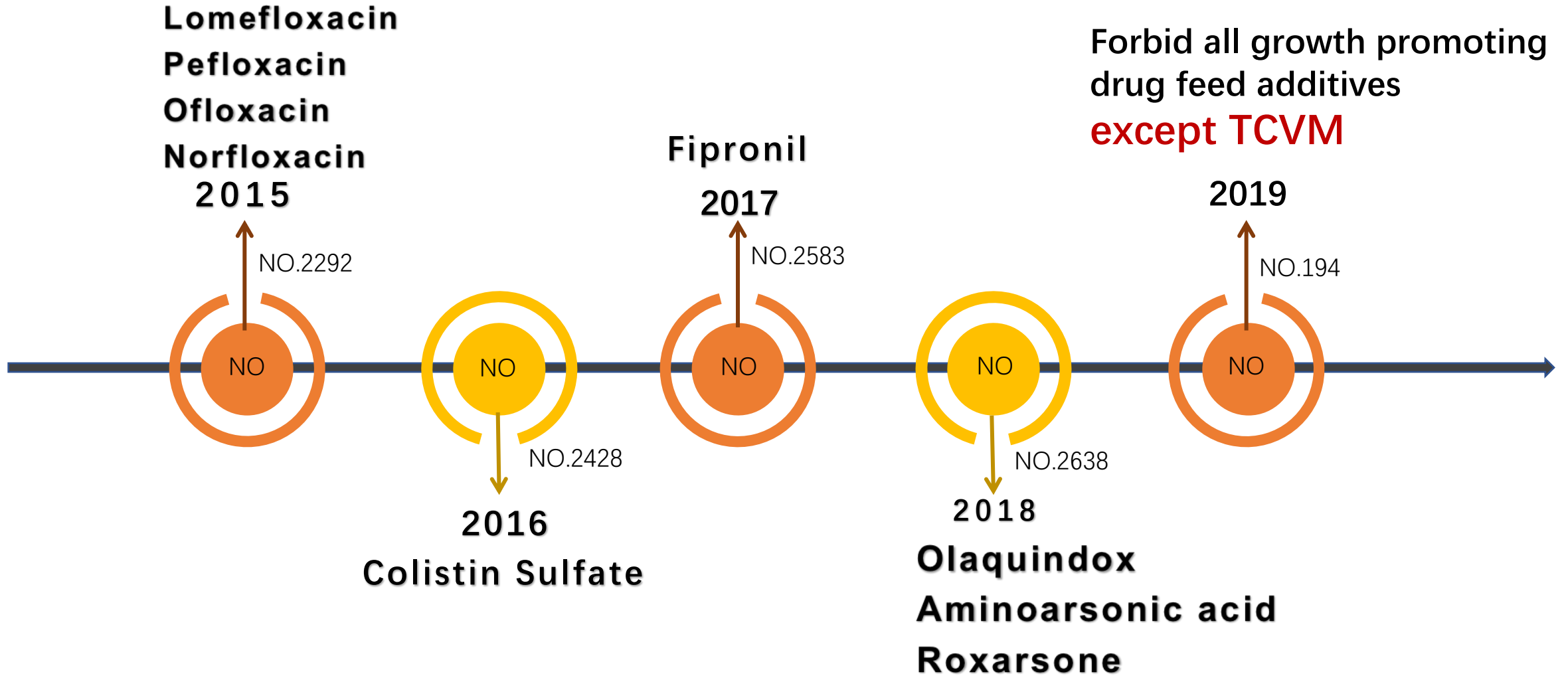
48%
(human)



52%
(animal)



Evolution of AGPs ban In China

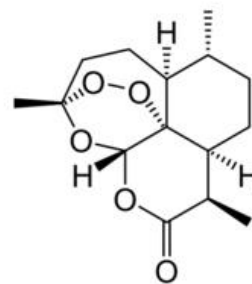


On November 2, 2017, the Ministry of Agriculture and Rural Affairs published the announcement on soliciting for the release of "catalogue of drug feed additives and specifications for their use" (Draft for comments), which made clear provisions on the use of drug feed additives.

序号	药物饲料添加剂名称	备注
1	二硝托胺预混剂	
2	土霉素钙预混剂	
3	山花黄芩提取物散 Scutellaria extract	新增
4	马度米星铵预混剂	新增
5	甲基盐霉素尼卡巴嗪预混剂	
6	甲基盐霉素预混剂	
7	吉他霉素预混剂	
8	地克珠利预混剂	
9	亚甲基水杨酸杆菌肽预混剂	新增
10	那西肽预混剂	
11	杆菌肽锌预混剂	
12	阿维拉霉素预混剂	新增
13	金霉素预混剂	
14	盐酸氨丙啉乙氧酰胺苯甲酯预混剂	
15	盐酸氨丙啉乙氧酰胺苯甲酯磺胺喹噁啉预混剂	
16	盐酸氯苯胍预混剂	
17	盐霉素预混剂	新增
18	盐霉素钠预混剂	
19	莫能菌素预混剂	
20	恩拉霉素预混剂	
21	海南霉素钠预混剂	
22	黄霉素预混剂	
23	维吉尼亚霉素预混剂	
24	博落回散 Macleaya cordata	新增
25	喹烯酮预混剂	新增
26	氯羟吡啶预混剂	



Youyou Tu was awarded the Nobel Prize in 2015 for her contribution to the discovery of artemisinin, a drug that has significantly reduced the mortality rates for patients suffering from malaria.



Artemisinin



Artemisia annua

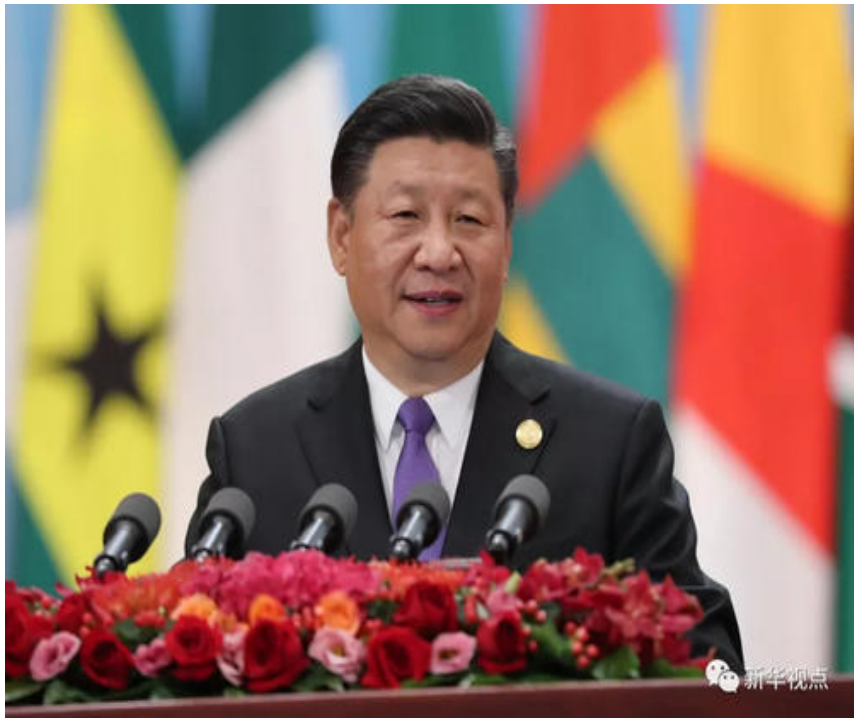
There are records in ancient medical books: take some artemisia annua and drink it's fresh juice to treat malaria.

“治疟疾寒热：青蒿一握，以水二升渍，绞取汁，尽服之”《肘后备急方》



Xi stresses role of traditional Chinese medicine for "Healthy China"

Source: Xinhua | 2019-10-25 16:37:21 | Editor: huaxia



October 25, 2019, Xi Jinping stressed that our country should carry on fine elements in TCM and innovate them, he said TCM is a treasure of Chinese civilization and underlined the efforts to promote TCM internationally and fully develop its unique strength in preventing and treating diseases.

TCM has been used for thousands of years and has many functions

Anti-viral

Anti-bacteria

Anti-tumor

Anti-cancer

Immune regulation

Growth Promote



Beijing Key Lab of Traditional Chinese Veterinary Medicine Beijing Engineering Technology Research Center of TCVM



Our Lab was founded in 2006, is the first provincial key laboratory engaged in the research of Chinese veterinary medicine. We focus on the following aspects of research:

- The mechanism of Acupuncture
- TCVM as feed additive
- TCVM treatment animal bacterial and viral disease



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北京市科学技术委员会
二〇一三年



Pulsatilla Decoction(PD), a classical traditional Chinese formula recorded in Treatise on Febrile Diseases (Shang Han Lun in 219 A.D) , has used to treatment diarrhea in piglets caused by *E.coli*.





Pulsatillae Radix
Baitou weng



Aash Bark
Qin Pi

Baitouweng Decoction
(Pulsatilla Decoction)



Coptidis Rhizoma
Huang Lian



Phellodendri Chinensis Cortex
Huang Pai

PD preparation

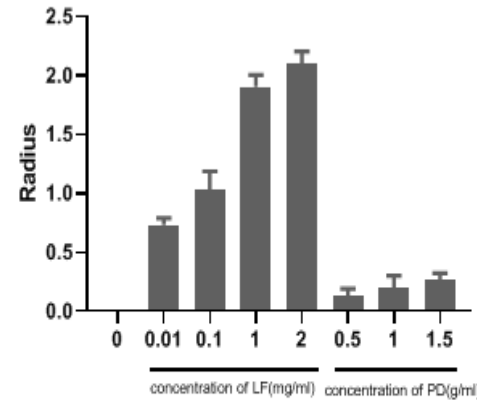
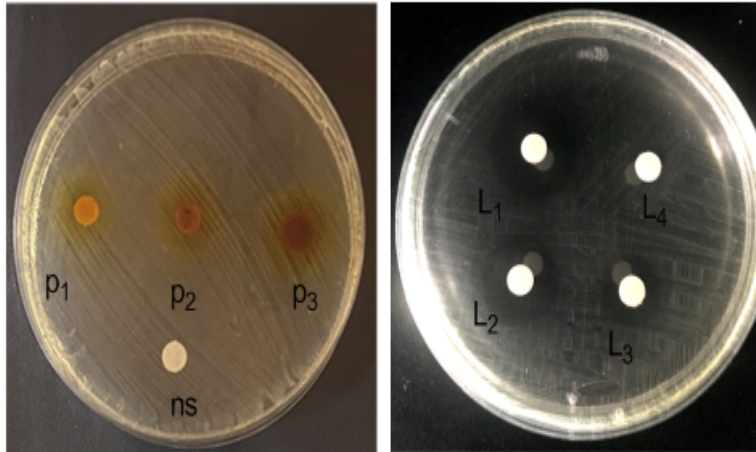


Water extract



Concentrate
(rotary evaporator)

Antibacterial test of PD in vitro



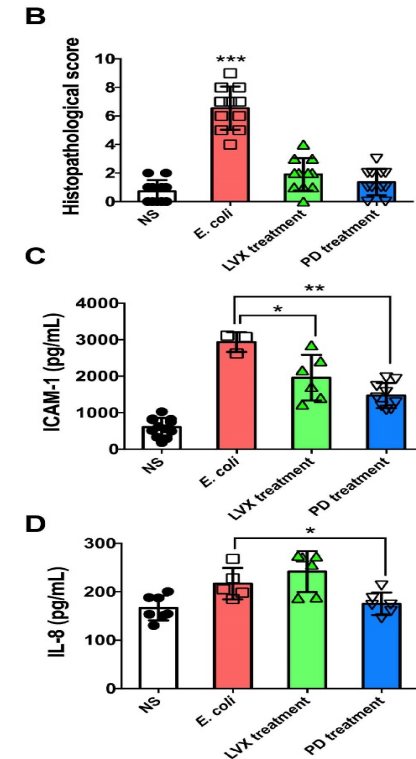
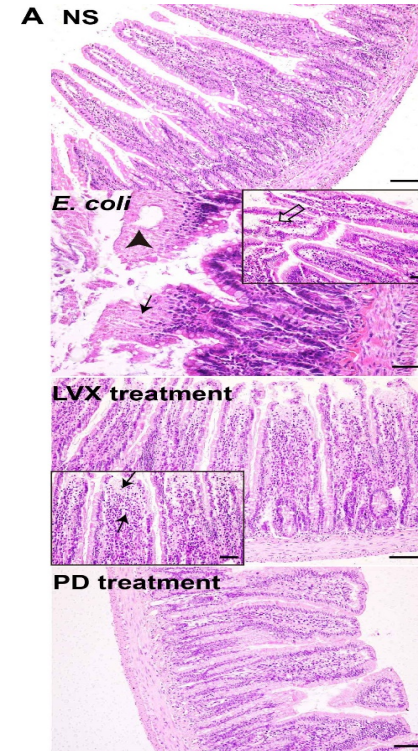
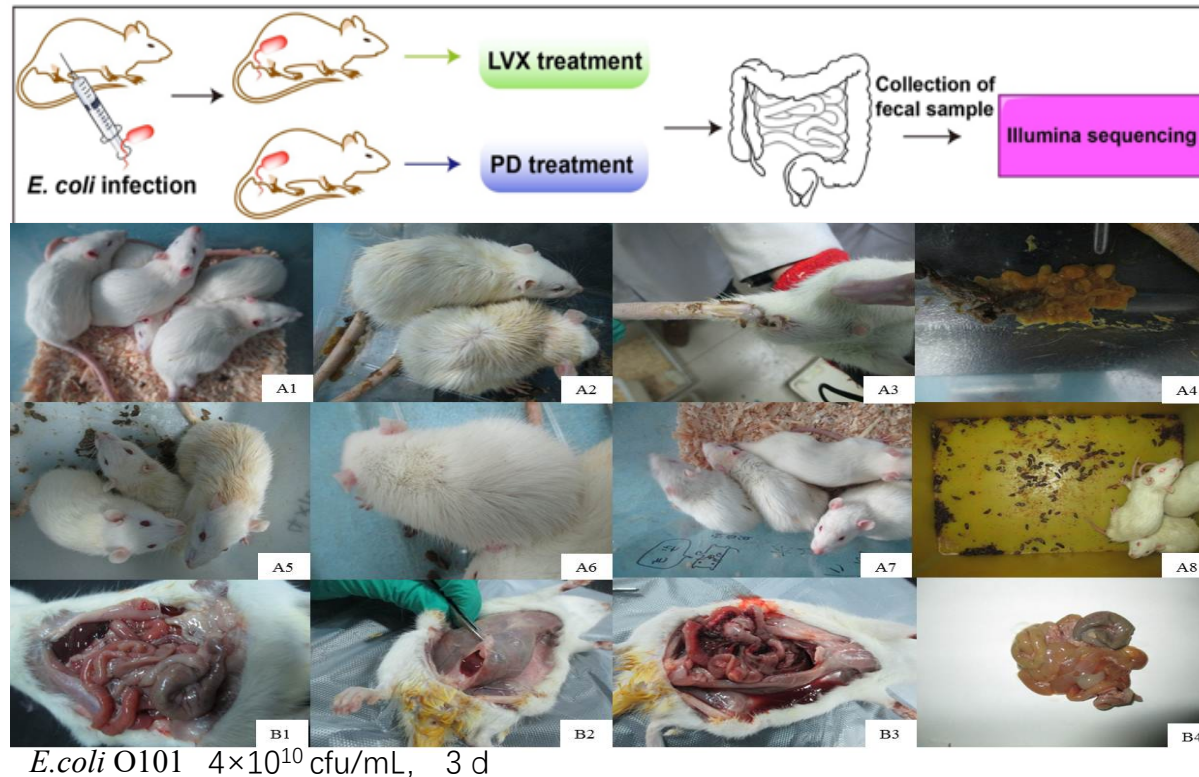
Pulsatilla Decoction and Levofloxacin Antibacterial Results

P₁:0.5g/ml Pulsatilla Decoction,P₂:1 g/ml Pulsatilla Decoction,P₃:1.5 g/ml Pulsatilla Decoction
L₁:2mg/ml Levofloxacin,L₂:1mg/ml Levofloxacin,L₃:0.1mg/ml Levofloxacin,L₄:0.01mg/ml
Levofloxacin, NS: Normal Saline.

Plate counting result shows that levofloxacin has obvious antibacterial activity effect on *E.coli*. While the PD of 0.5, 1.0 and 1.5g/mL have no obvious antibacterial activity effect on *E.coli*.

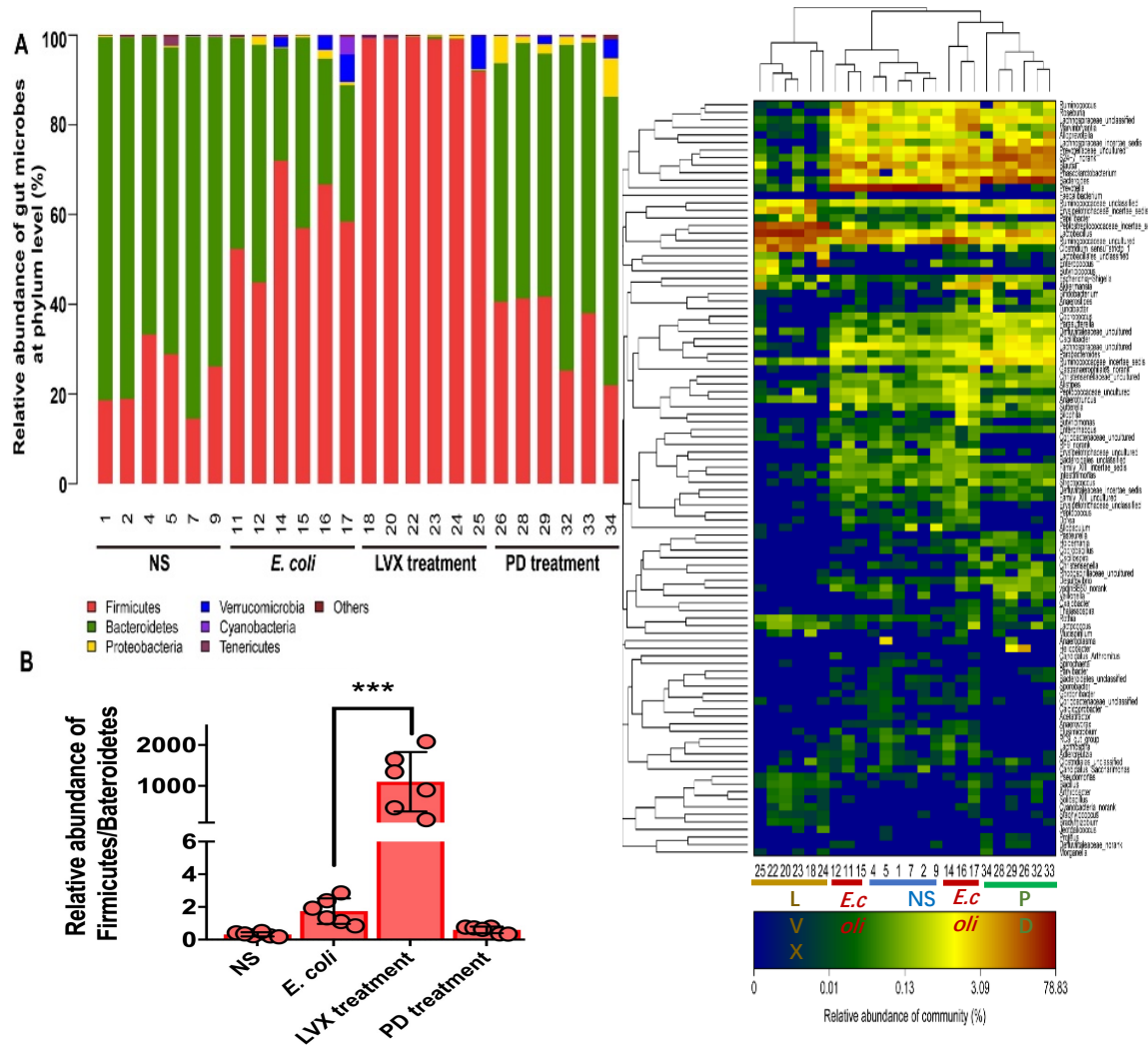
PD can not directly kill bacteria in vitro

Antibacterial test of PD in vivo



- From the clinical symptoms, PD and Levofloxacin(LVX) can reduce the soft stool rate of rats.
- HE staining assay show that the treatment of PD and LVX can alleviate the intestinal lesions, but the inflammatory cell infiltration still exists in LVX group.
- PD treatment also decreased Interleukin-8 and Intercellular Adhesion Molecule-1(ICAM-1).
- PD and LVX (Levofloxacin) could treat diarrhea caused by *E. coli* in rats.

Intestinal microflora

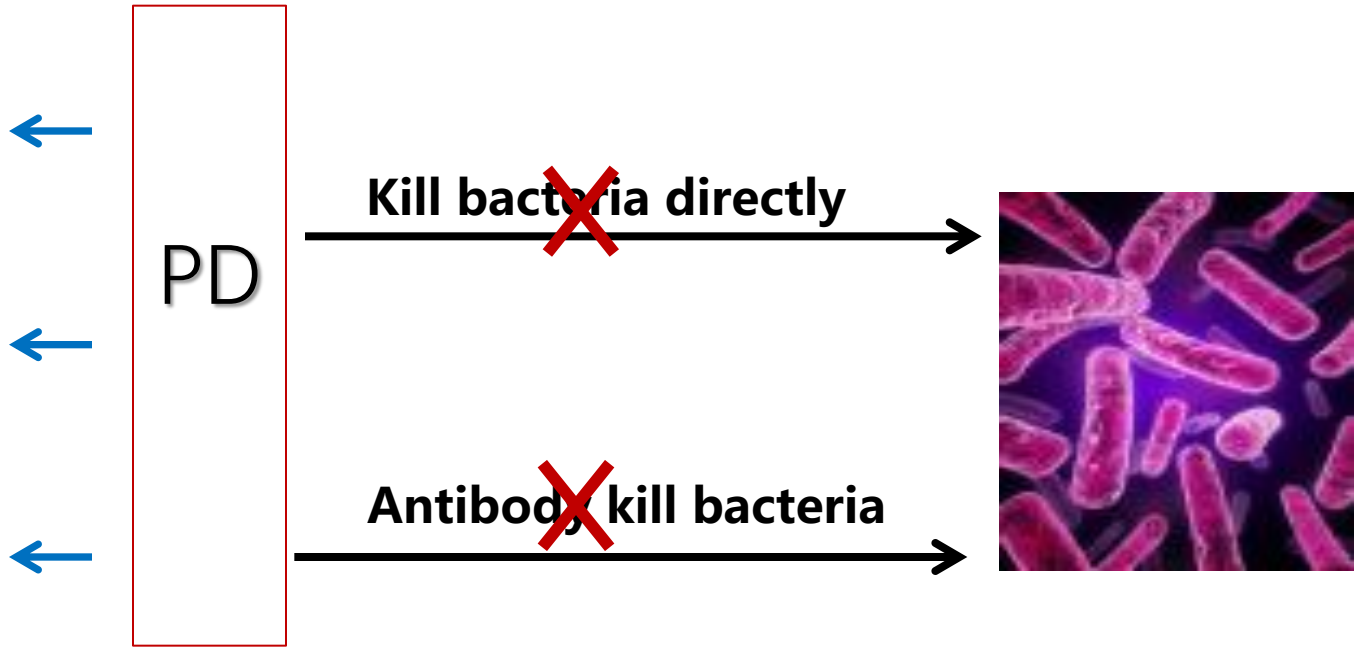


- 16S rRNA was used to analyze the intestinal microflora of rat feces
- In normal rats, the Firmicutes/Bacteroidetes (B/F) ratio is 3.245, *E. coli* infection can decrease the Bacteroidetes, so the ratio is 0.644. Treatment with PD, increase in the relative abundance of Bacteroidetes, and the B/F ratio is 1.732, But after treatment with LVX, the Bacteroidetes decreased rapidly and the ratio of B/F in antibiotic group is 0.002.
- We also found that after the treatment PD, no *E. coli* intraperitoneal injection was found in the feces.

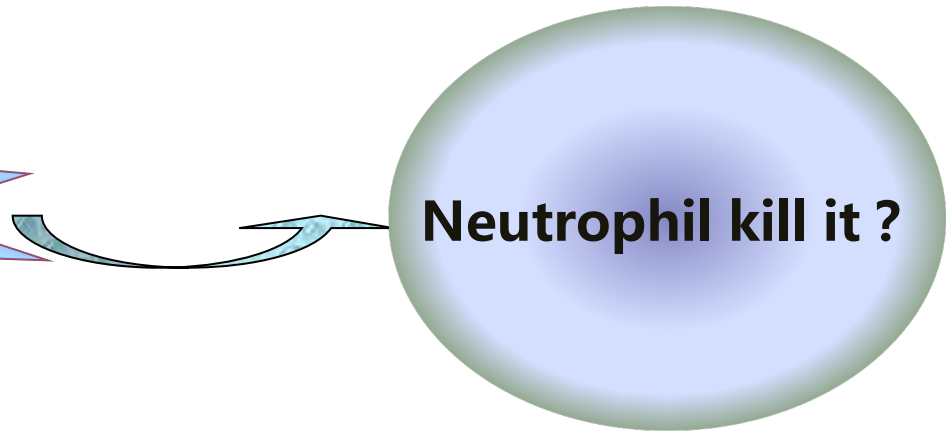
Can not prevent bacterial growth in vitro,
and the blood concentration has no
effect on killing bacteria

No pathogenic bacteria can be isolated
in the body after TCM treatment

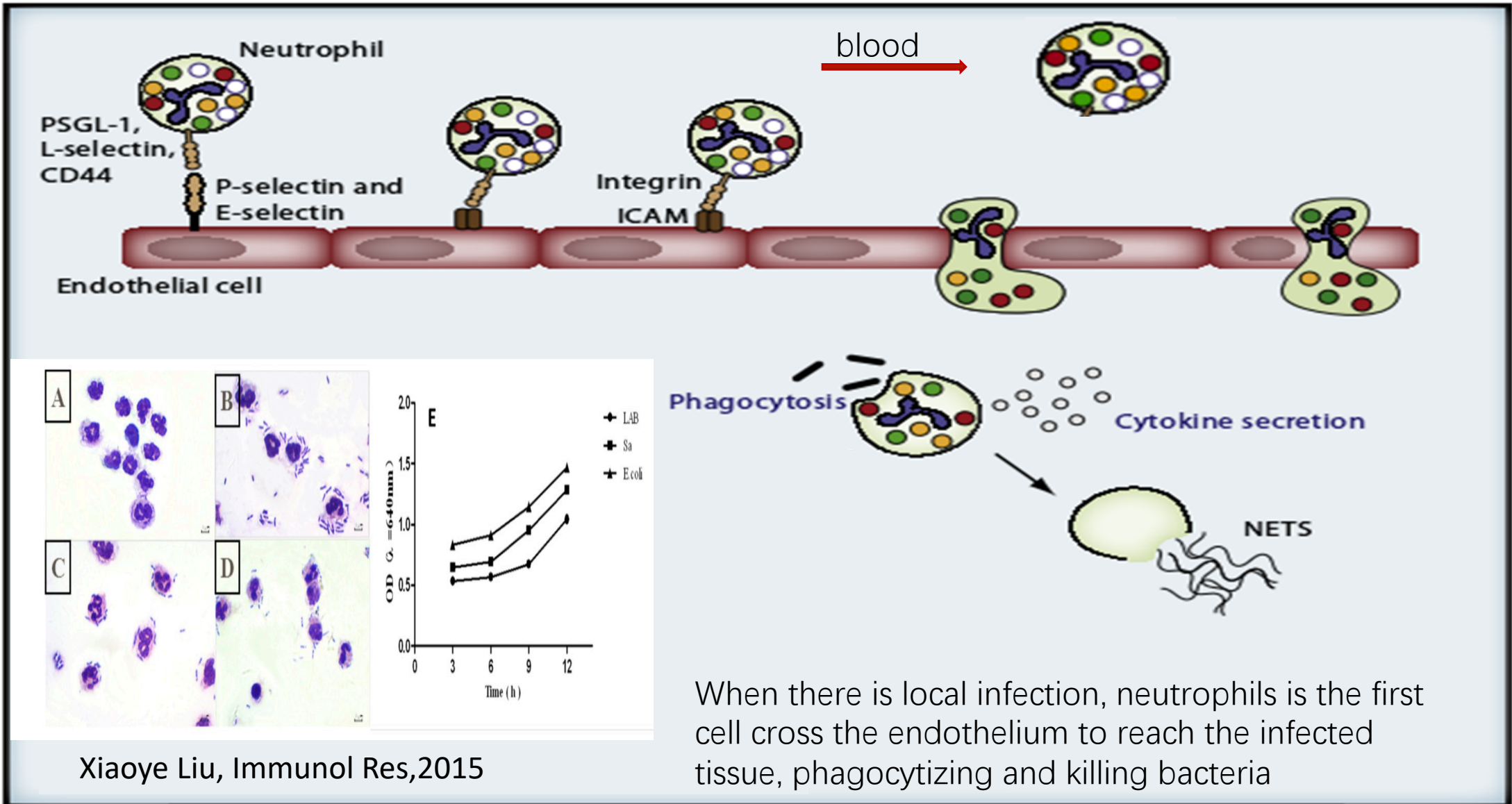
The treatment time of TCM is shorter
than that of antibody production in vivo



Where is the bacteria



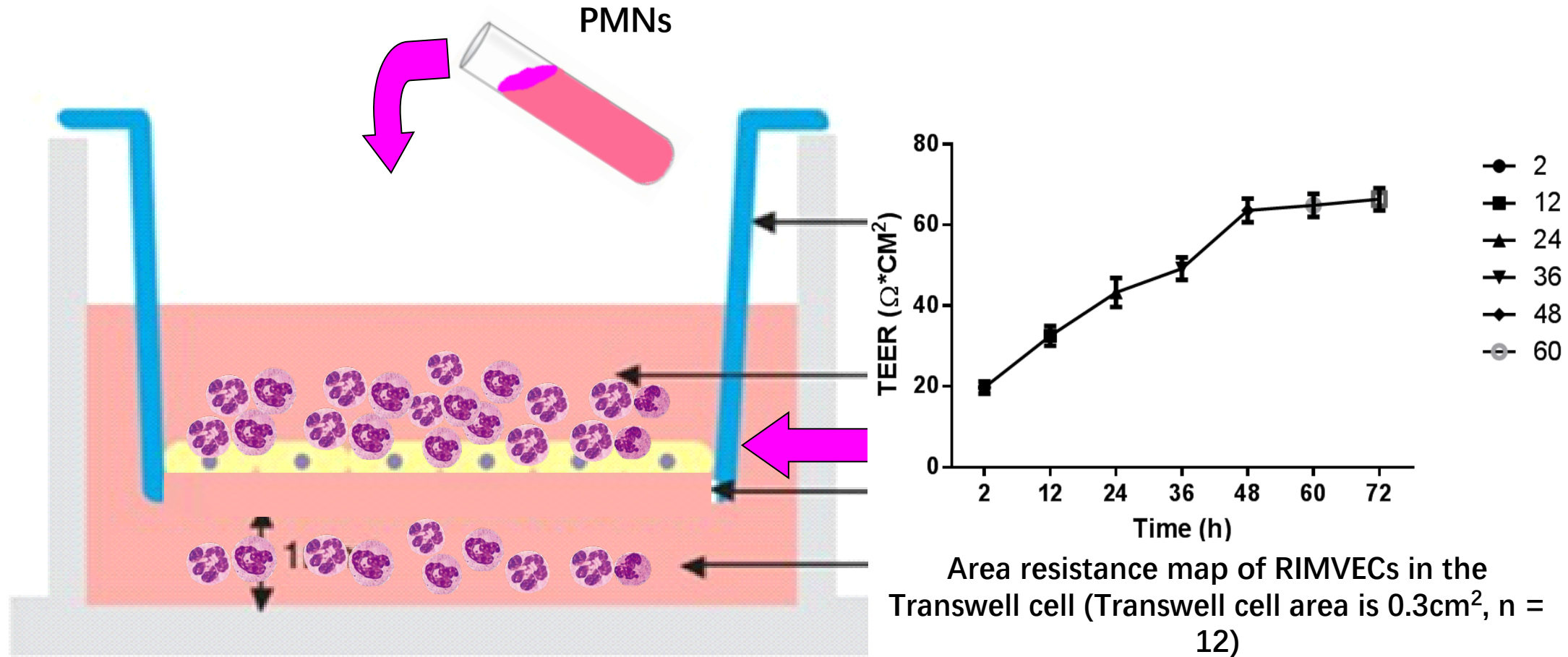
PD may remove the infected bacteria by mobilizing the function of Neutrophil(PMN)



Xiaoye Liu, Immunol Res, 2015

Endothelial cell is important for neutrophil to kill bacteria



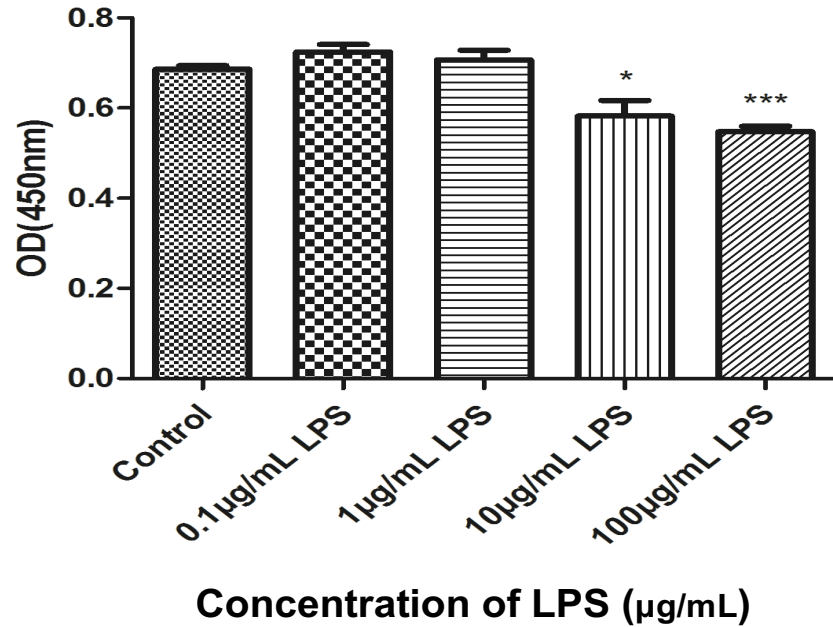
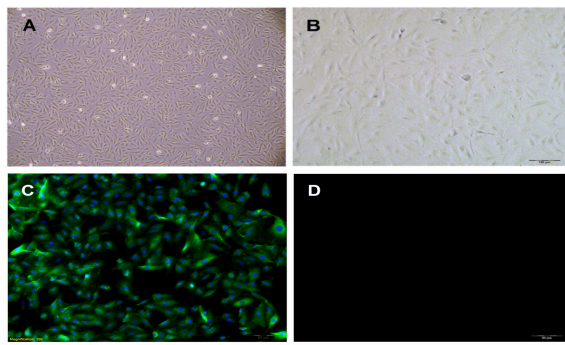


- Established PMNs / RIMVECs transwell model using polycarbonate (PC) membrane with 5μm pore size
- Measured the transmembrane resistance (Teer) of monolayer cells to detect whether the endothelial cells cover the whole plate

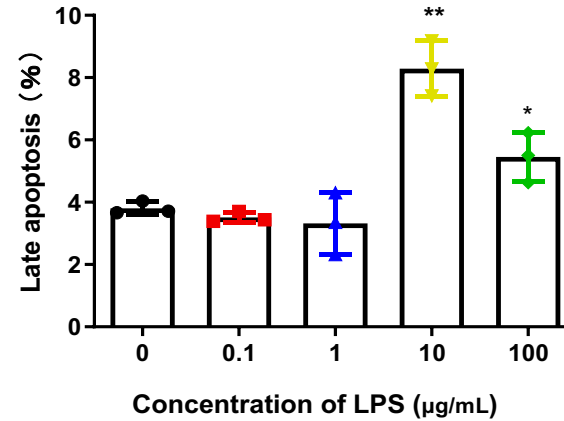
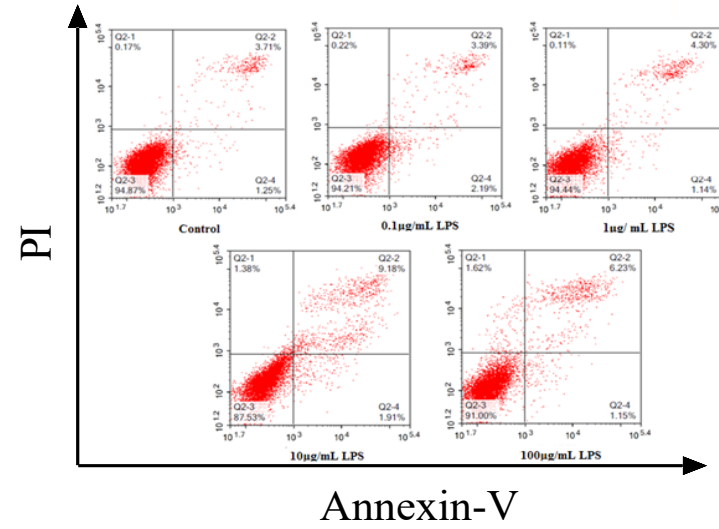
Establishment of Endothelial Injury Model



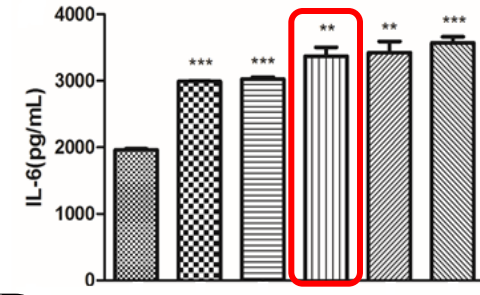
A



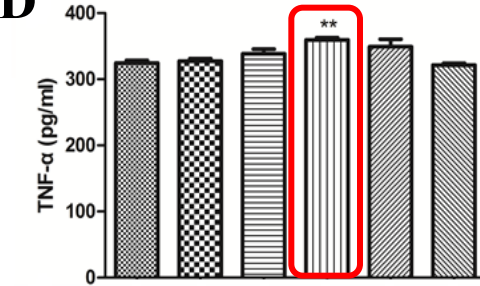
B



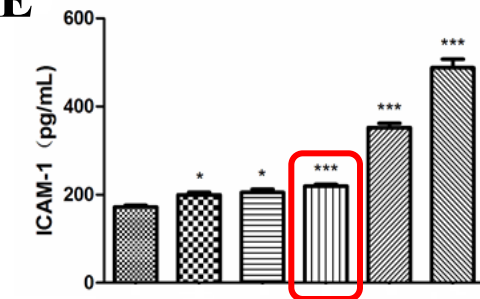
C



D



E

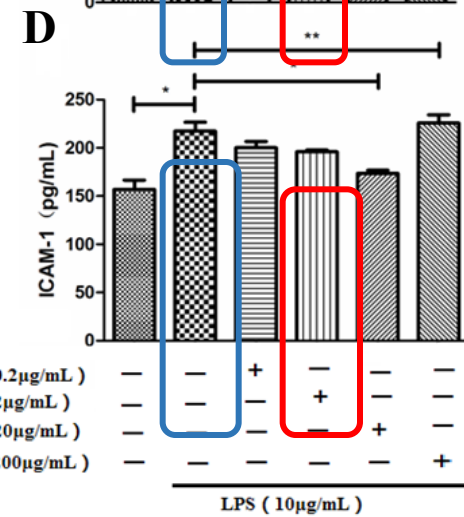
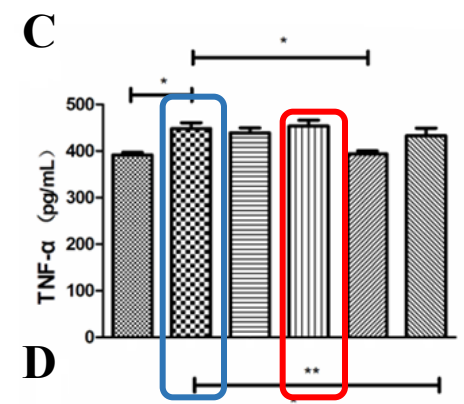
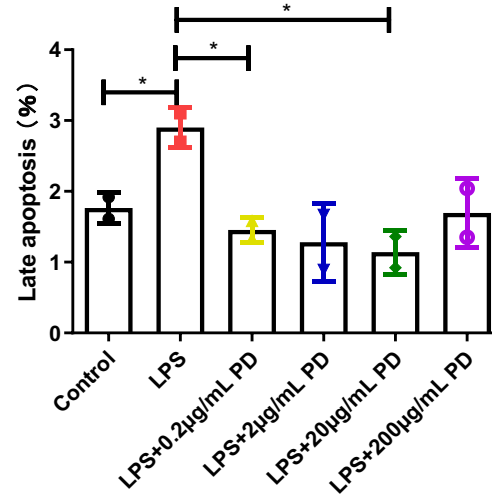
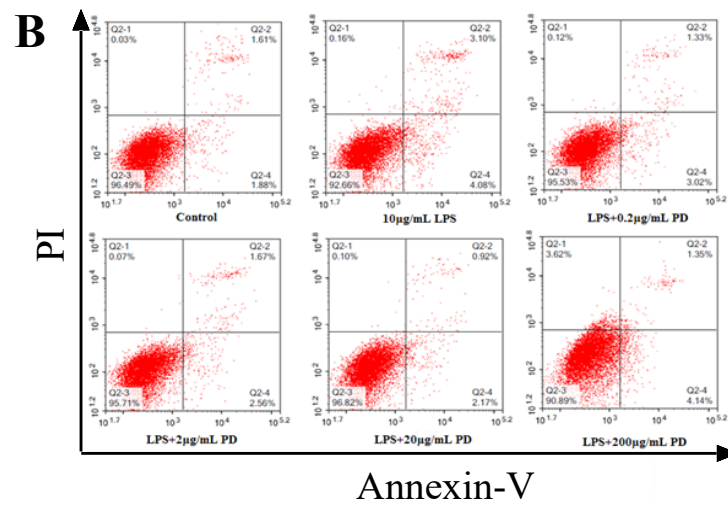
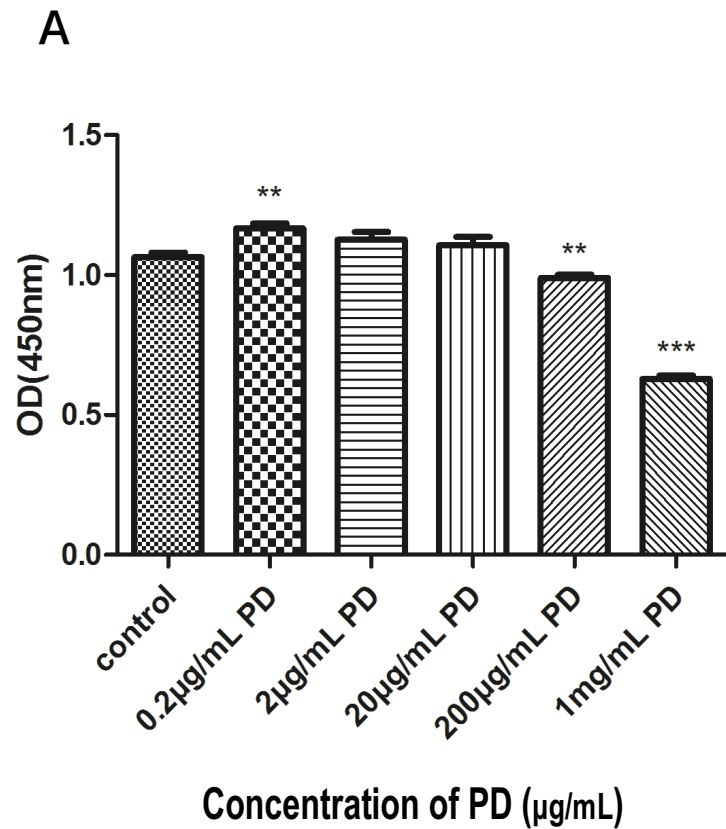


LPS (0.1μg/mL)	-	+	-	-	-
LPS (1μg/mL)	-	-	+	-	-
LPS (10μg/mL)	-	-	-	+	-
LPS (50μg/mL)	-	-	-	-	+
LPS (100μg/mL)	-	-	-	-	+

10 μg/mL LPS has significant cytotoxicity to RIMVCs, can significantly increased the expression level of IL-6, TNF - α and ICAM-1, and induce cell apoptosis in the late stage. The injury model of RIMVCs induced by LPS was successfully established.

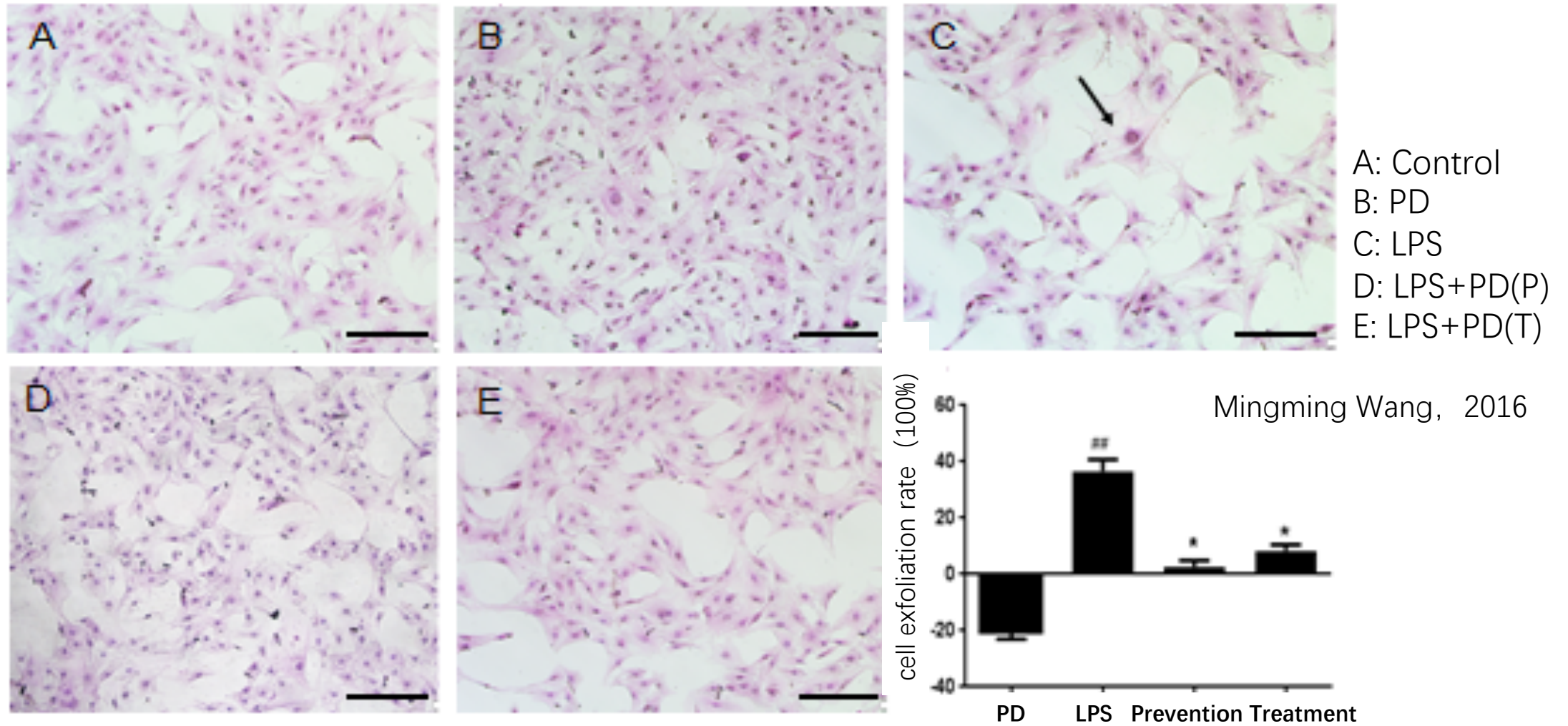
Evaluation of the protective effect of PD on endothelial cell



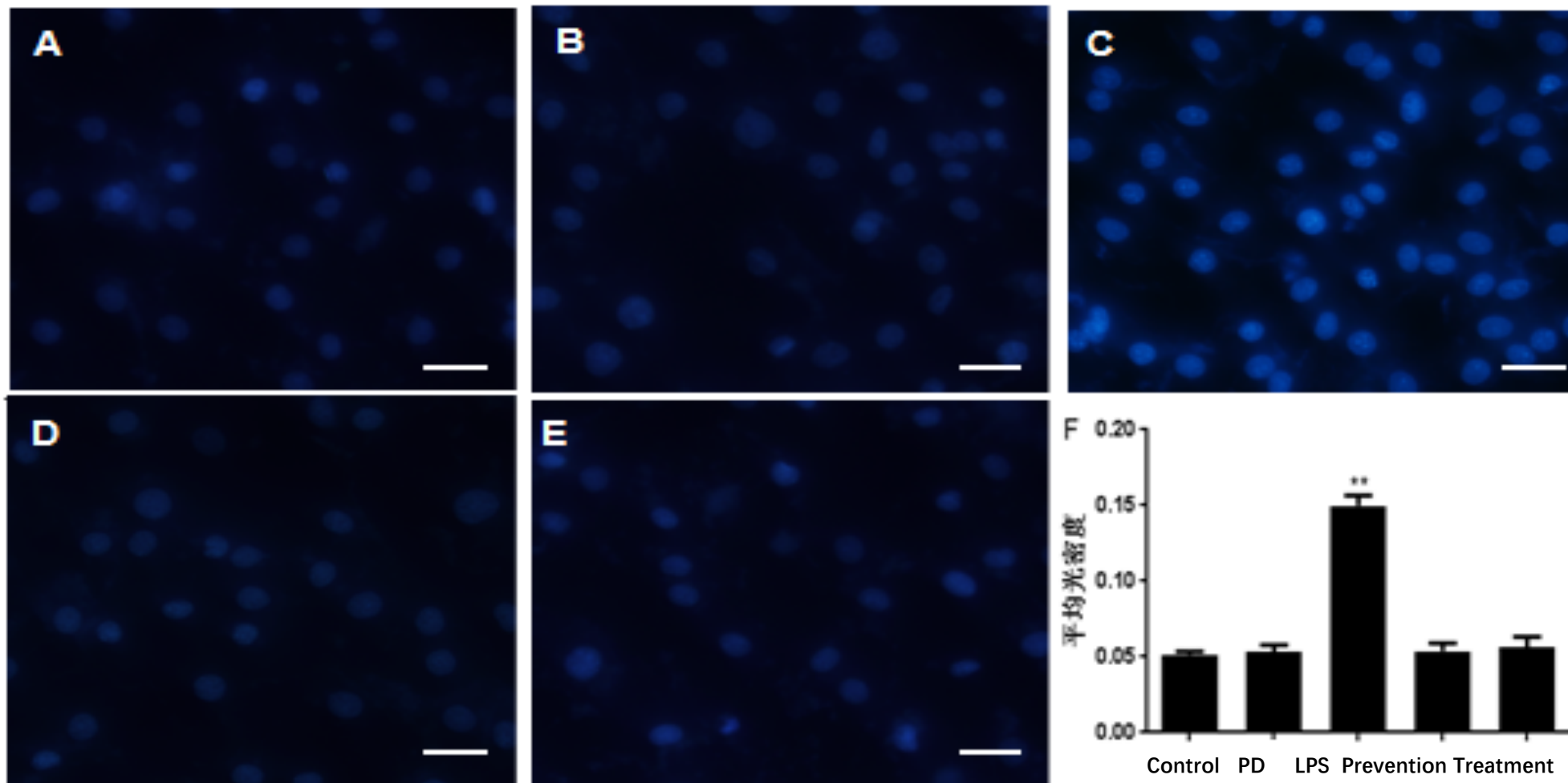


PD (0.2 $\mu\text{g/mL}$)	PD (2 $\mu\text{g/mL}$)	PD (20 $\mu\text{g/mL}$)	PD (200 $\mu\text{g/mL}$)	LPS (10 $\mu\text{g/mL}$)
-	-	-	-	-
-	-	-	-	+
-	-	-	+	-
-	-	+	-	-
-	-	-	-	+

Less than 20 $\mu\text{g/mL}$ PD has no cytotoxicity to RIMVECs, and it could improve the apoptosis of RIMVECs induced by LPS, and significantly reduce the expression of TNF- α and ICAM-1, so PD can play a good protective role for RIMVECs.



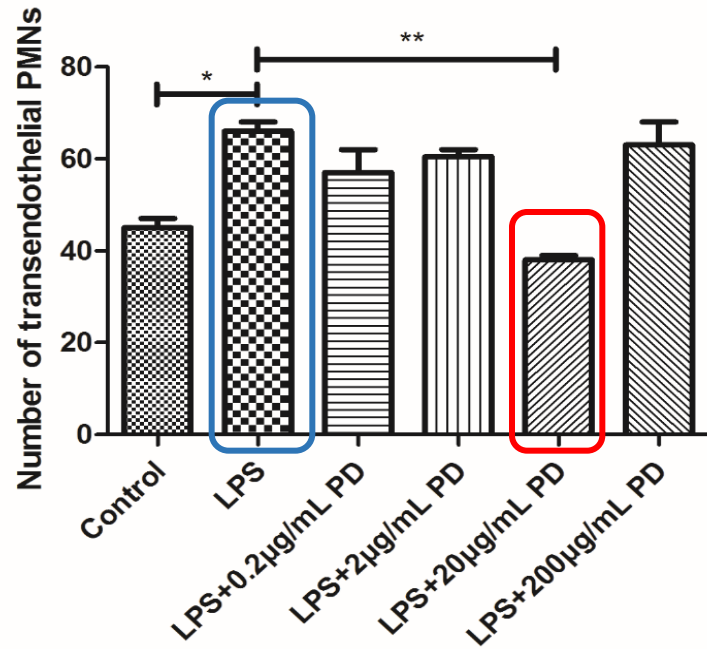
Cell exfoliation rate was used to evaluate the injury of endothelial cell and the protective effect of TCVM. HE staining results showed that the number of cells in PD group increased and no cell fell, but in LPS group, the cells were stretched and a large number of cells fell off, the rate was 39.6%. The rate in the prevention and the treatment group were significantly lower than that in the LPS group.



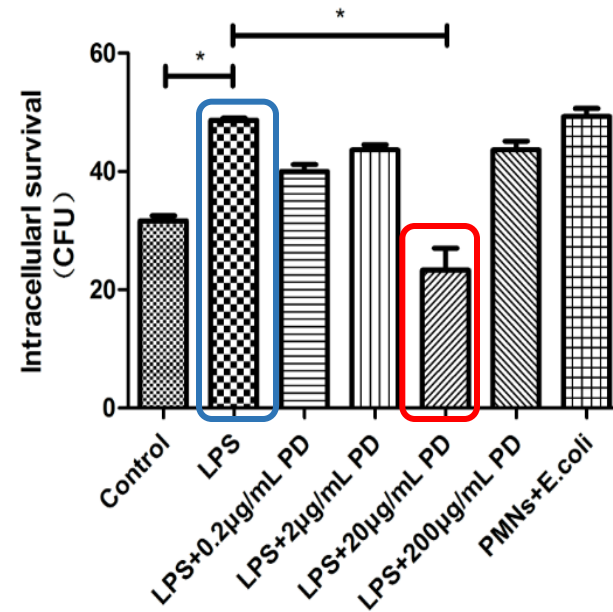
Mingming Wang, 2016

When the cells were damaged, dense particles and fragments of DNA fluorescence could be seen in the nucleus or cytoplasm. The results showed that LPS could damage the membrane and nucleus of microvascular endothelial cells, and PD could reduce the damage caused by LPS.

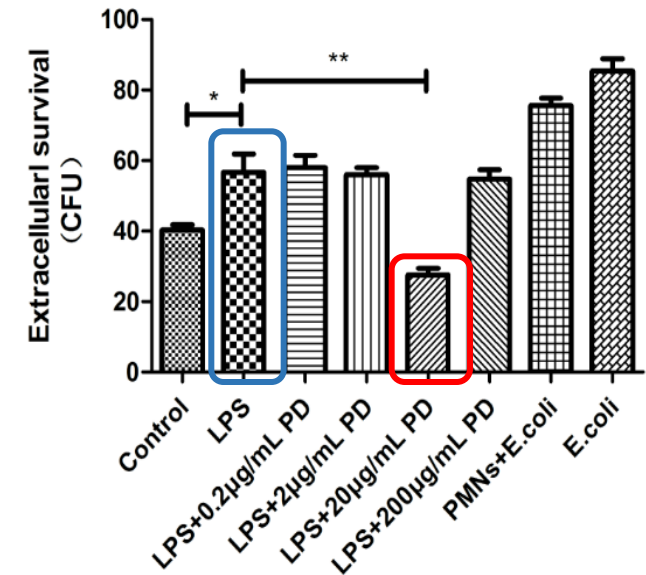
Transendothelial PMNs kill the *E.coli*



Number of transendothelial PMNs

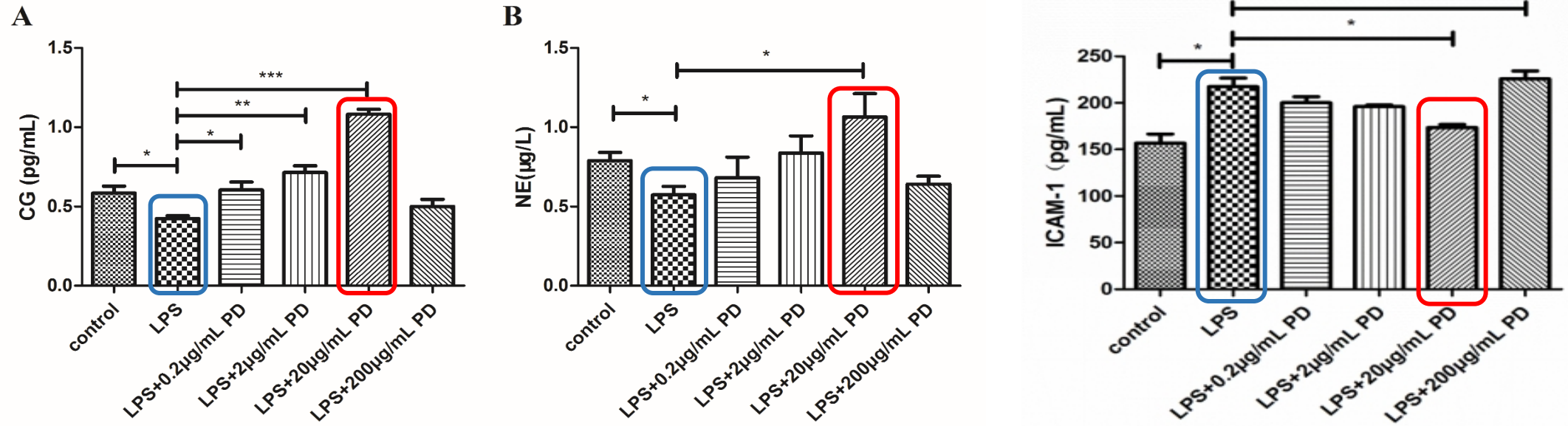


Kill bacterial function of PMNs



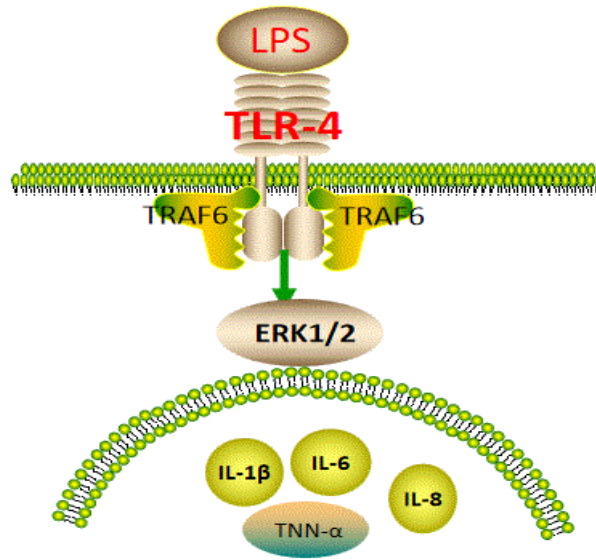
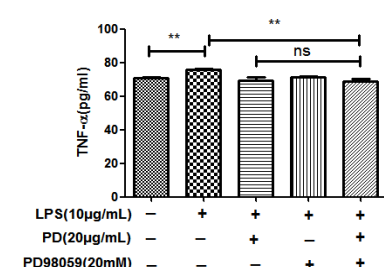
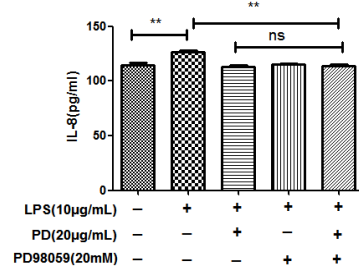
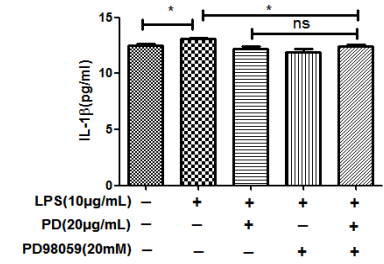
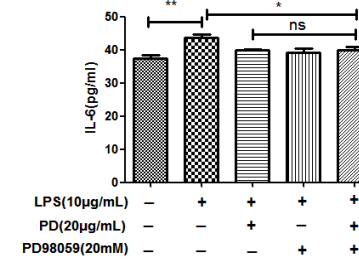
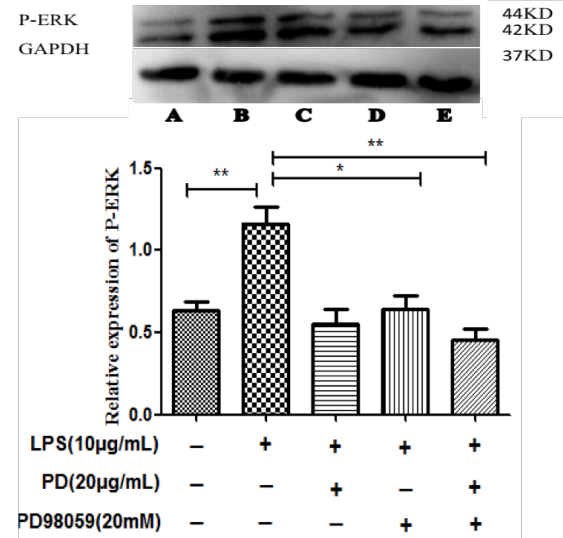
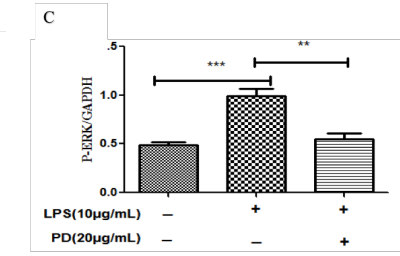
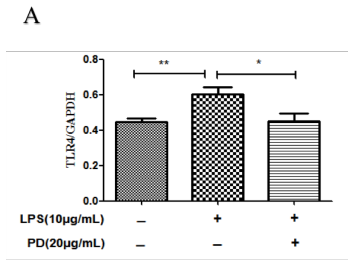
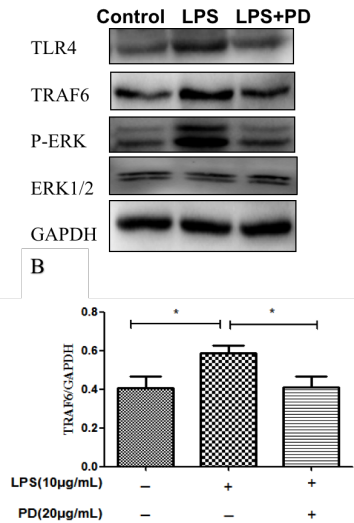
The results showed that the number of neutrophils across the endothelium increased after LPS injury, while the number of neutrophils across the endothelium decreased after Chinese medicine treatment. Although neutrophils are reduced, their kill bacterial function is increased.

Detection of NE , CG and ICAM-1 released from transendothelial PMNs



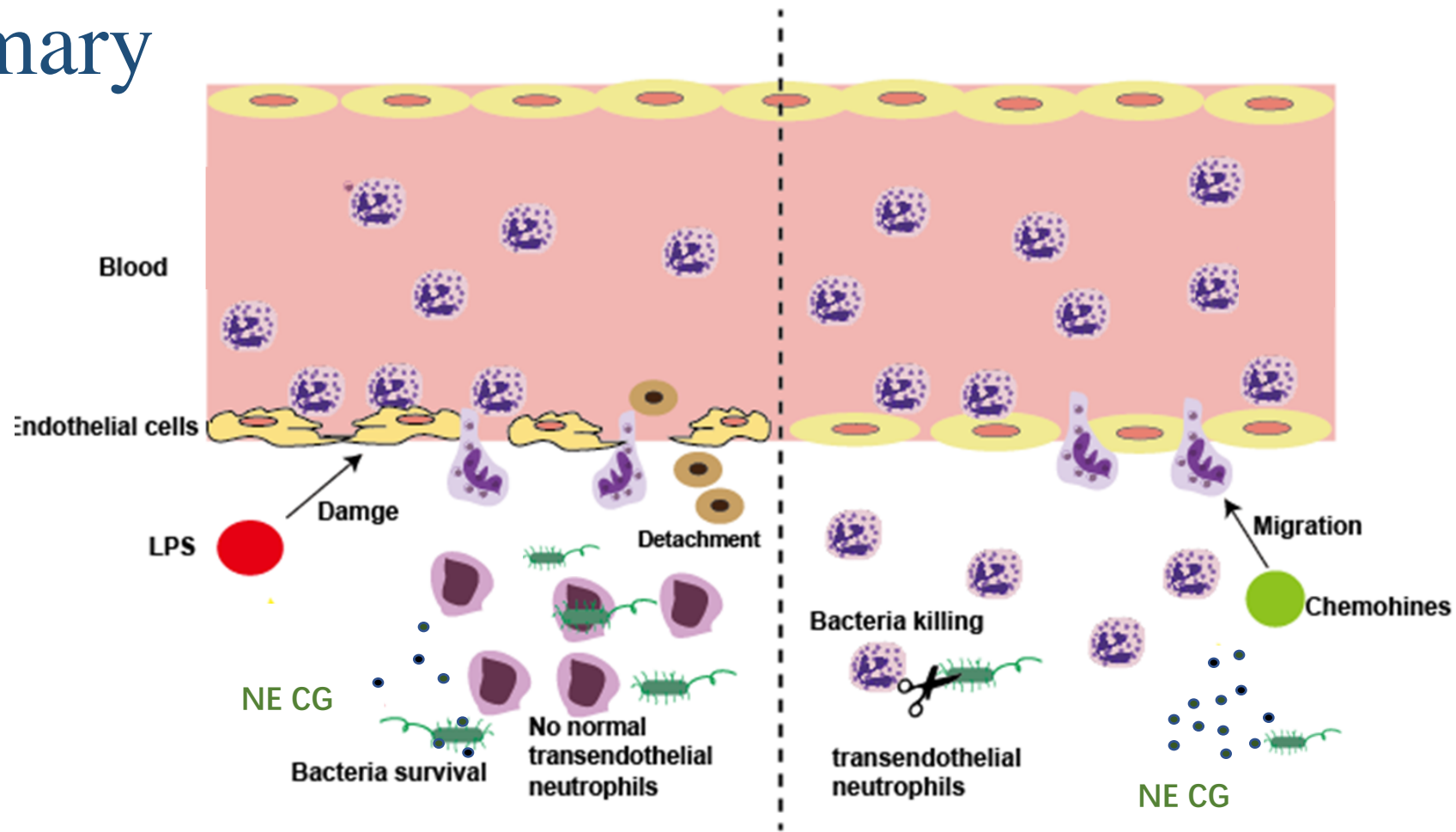
Intercellular cell adhesion molecules-1 (ICAM-1) was increased in LPS group, and decreased in PD group, which also explained the reason of the increase number of PMN crossing the endothelial cell in LPS.

Elastase (NE) and Cathepsin G (CG) is released by PMN, when treatment with PD, the NE and CG increased to kill the bacterial.

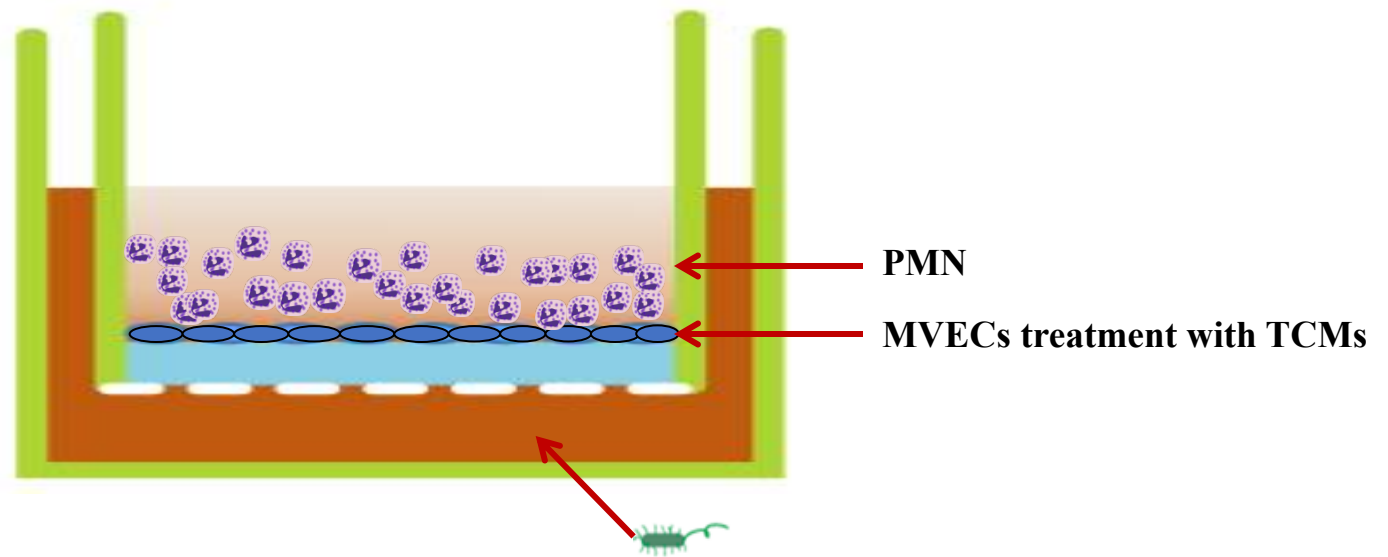


PD may protect RIMVEs from LPS injury by reducing the expression of TLR-4 and TRAF6 protein, inhibiting the activation of p-ERK and reducing the secretion of inflammatory factors IL-6, IL-8, IL-1 β and TNF - α , so as to play an anti-inflammatory role.

Summary

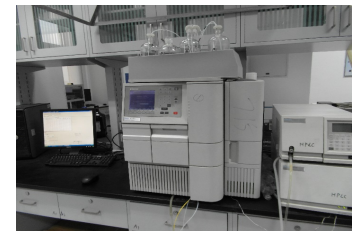


1. Neutrophil cross the integrated microvascular endothelial cells (MVECs) can kill the bacteria
2. When cross the integrated MVECs, Neutrophil can release the NE and CG to kill the bacteria
3. PD is to protect endothelial cells from LPS damage and mobilize Neutrophil to kill bacteria, like the words in china "fu zheng qu xie" means "Strengthening the body resistance to eliminate pathogenic factors"

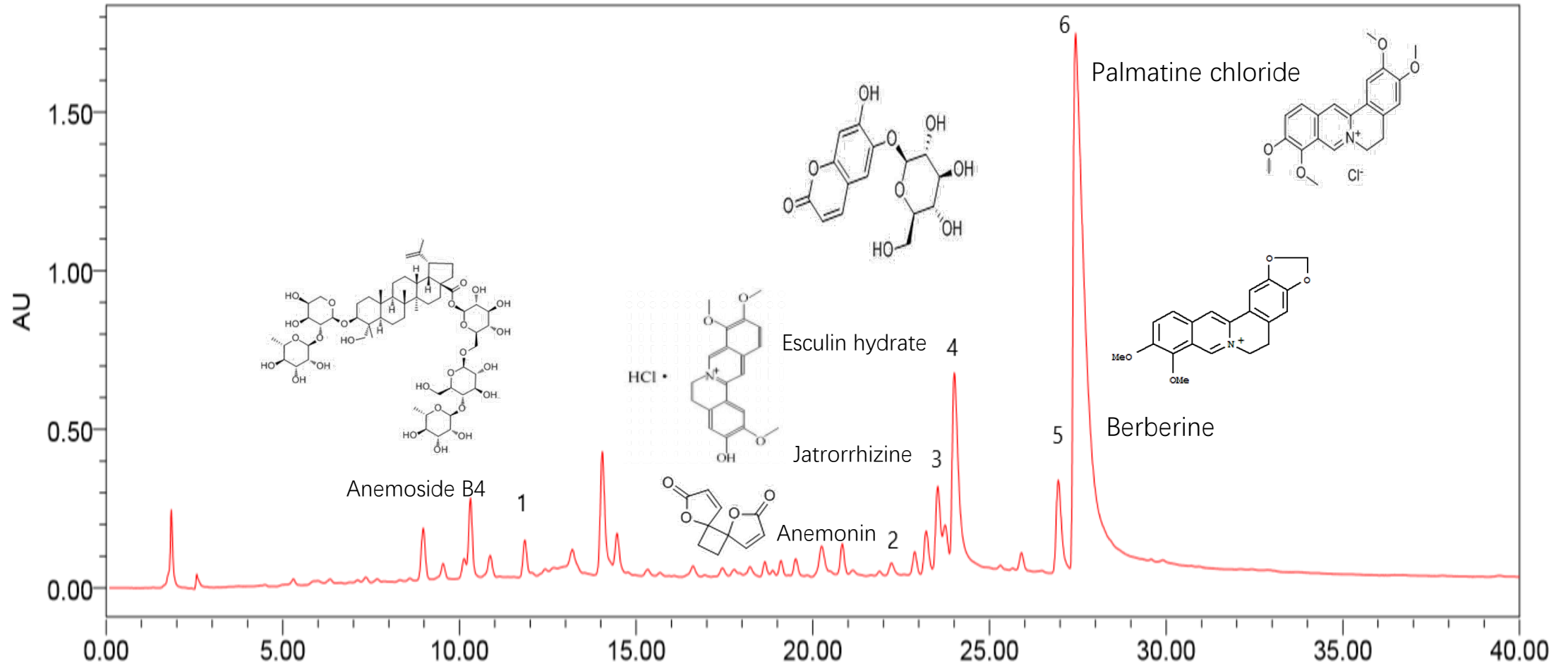


We have established a model for screening antibacterial TCM in vitro

PD components



HPLC



HPLC SampleName PD; Vial 1; Injection 1; Channel 2998 Ch1 220nm@1.2nm; DateAcquired

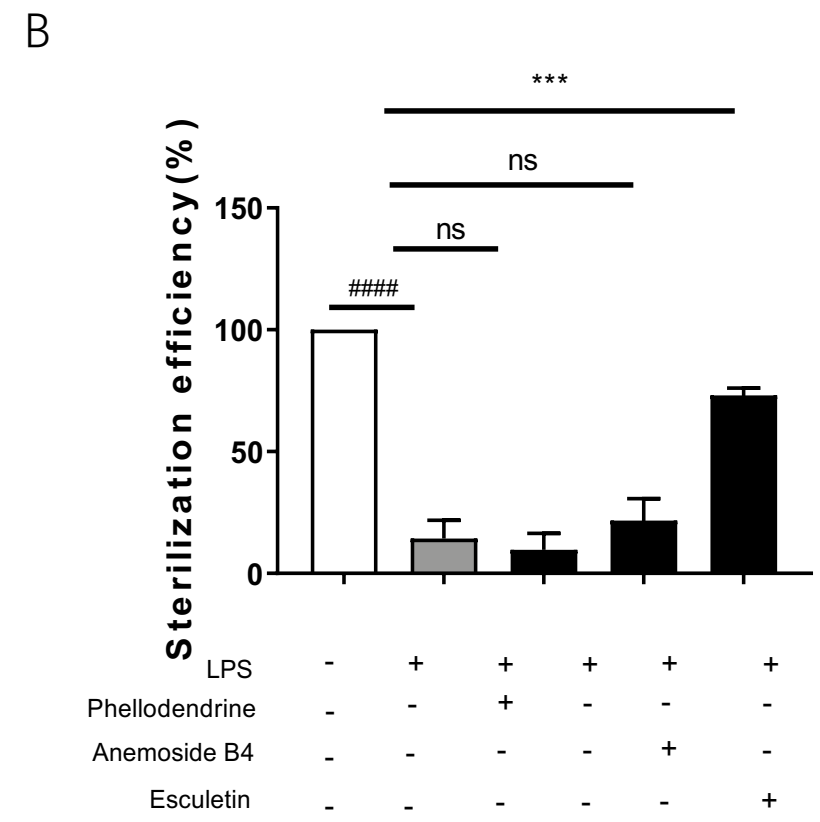
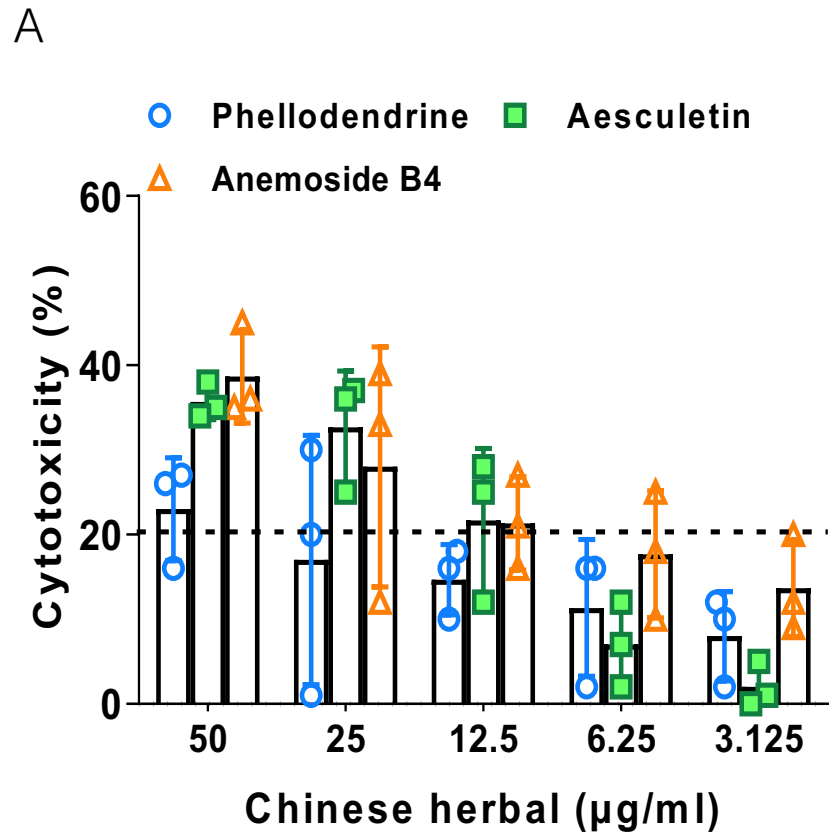


Figure. (A) Cytotoxicity of RI cells treated with Aesculetin, Anemoside B4 and Phellodendrine. (B) Sterilization efficiency of Aesculetin, Anemoside B4 and Phellodendrine.


The results that stimulation of RIMVCs cells by LPS will reduce the anti-bacteria of PMNs, but it can significantly restore the anti-bacterial efficiency of PMNs after treatment with a Esculetin.

TCVM feed additive used to weaned piglets



			21d- 51d
Group	TCVM	Chlortetracycline	A company
FCR	1.382	1.414	1.444
Mortality	0	0	6.25%

The feed conversion ratio in TCVM group was basically the same as that of antibiotics, and even lower than it, the mortality is 6.25% when use a company feed additive.



Traditional Chinese medicine plays an exact role in growth promoting and diseases treatment. With the help of the government 's attention and modern scientific methods, the application of traditional Chinese medicine will have a good prospect.

Acknowledgement



Shangwen He



Xin Wang



Yun Wang



THANK YOU

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