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# NF-κB in mitochondria regulates PC12 cell apoptosis following lipopolysaccharide-induced injury

**Key words:** **Nuclear transcription factor κB (NF-κB), Mitochondria, Apoptosis, Adenine nucleotide translocase 1 (ANT1), Lipopolysaccharide (LPS)**

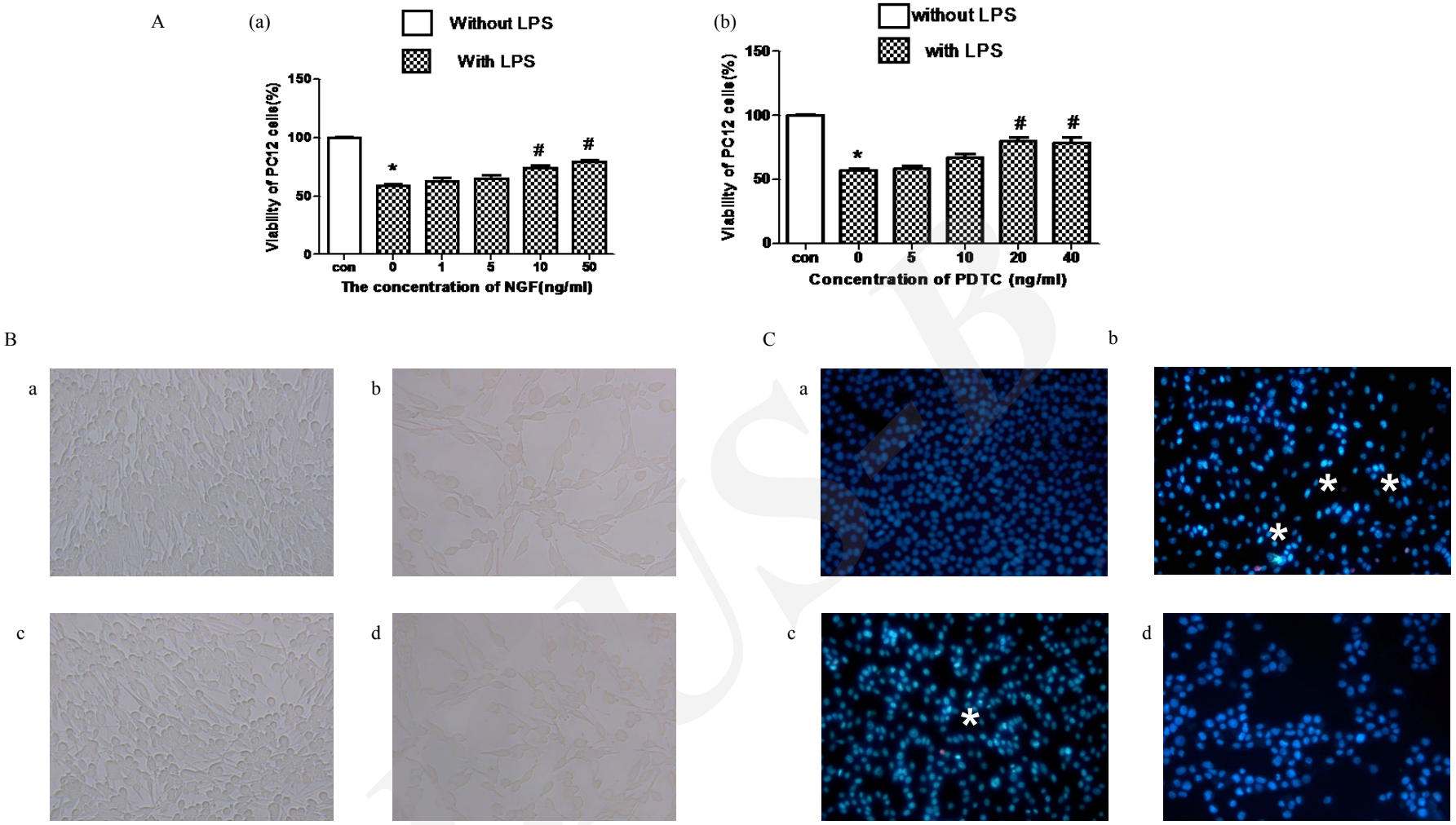



**Fig. 1 The effect of nerve growth factor and PDTC on PC12 cells.**

Fig.1A-a, NGF alleviated PC12 cells from LPS injury. Fig. 1A-b, PDTC alleviated PC12 cells from LPS injury.

Fig. 1B,C The effect of PDTC and NGF on LPS injury under the microscope.

(B) Cell morphology was observed by inverted microscope (×400). (a) Control: the differentiation of PC12 cell was spindle or oval-shaped, which distributed as clusters; (b) LPS: the cells shrink, and the synapses disappear; (c) LPS+ NGF and (d) LPS+ PDTC. If adding PDTC or NGF with LPS simultaneously, the damage reduced.

(C) Cell morphology observation by fluorescence microscope after Hoechst33342 staining. (a) Control: The nucleus of normal PC12 cells was blue by Hoechst33342 staining under fluorescence microscope; (b)LPS: The cells' nucleus condensed, and have come into the apoptosis process; (c) LPS+ NGF; (d) LPS+PDTC: The apoptosis cells significantly reduced in PDTC or NGF group.

Data are expressed mean ± SD. *$P < 0.01$ vs. control; #$p < 0.05$ vs. LPS injury groups.

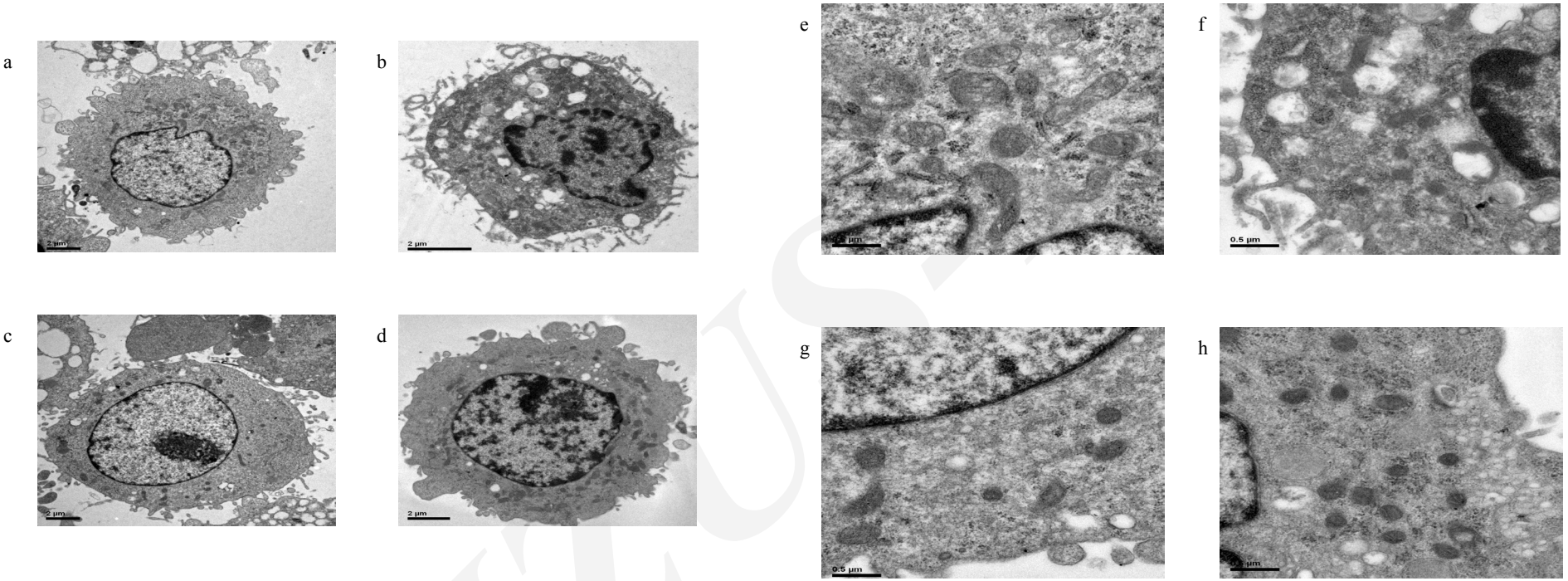



**Fig. 2 Subcellular morphology observed under transmission electron microscope.**

Fig. 2-a: Normal groups of PC12 cells showed clear intact nucleus; 2-b: LPS-induced apoptosis, PC12 cell's nucleonic heterochromatin condensed; 2-c, d: In the presence of NGF or PDTC, PC12 cells maintained relatively normal nuclear morphology.

Fig. 2-e: Normal mitochondria of PC12 cells; 2-f: LPS-induced apoptosis, PC12 cell's mitochondrial membrane appears with cavity and its matrix disappeared; 2-g, h: In the presence of NGF or PDTC, PC12 cells maintained relatively normal mitochondrial morphology.

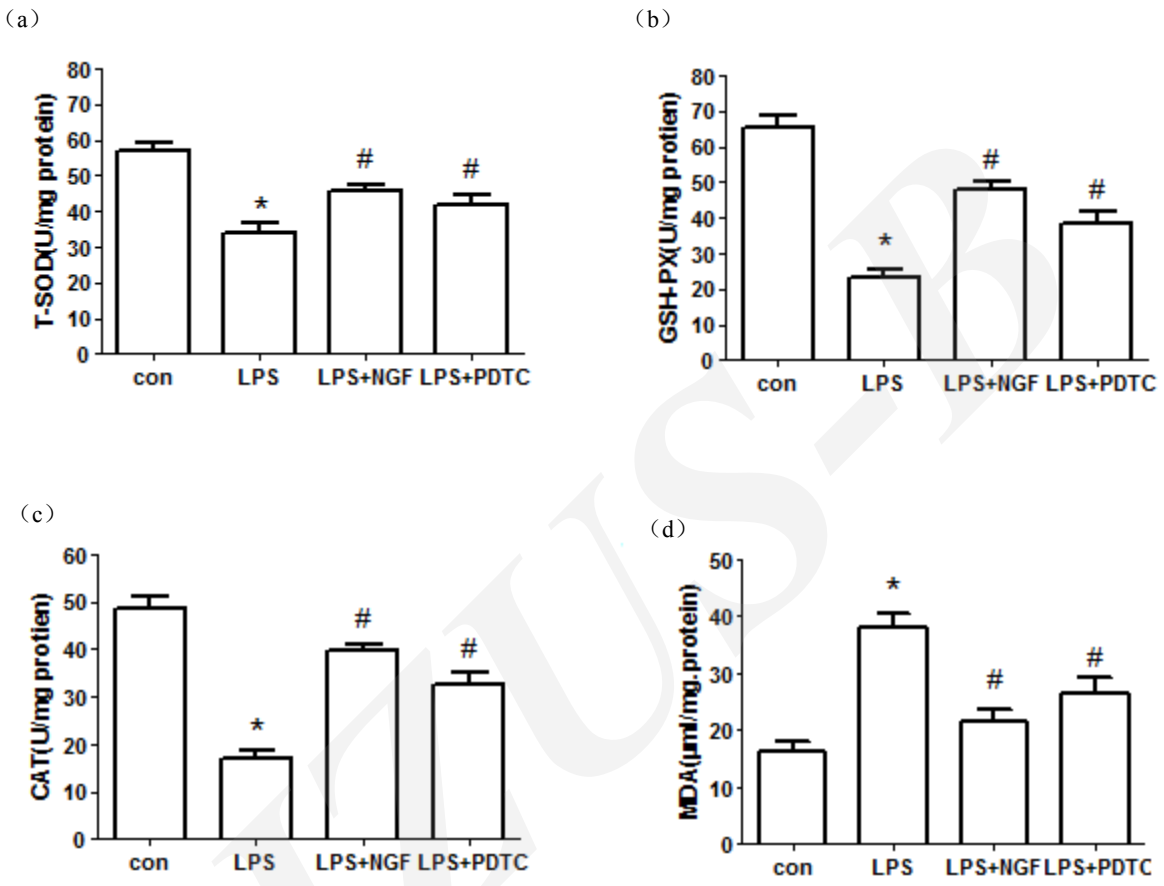



**Fig. 3 The effect of NGF and PDTC on PC12 cells by determining the activity of oxidation and anti-oxidation enzyme.** (a)SOD; (b) GSH-PX; (c) CAT; (d) MAD. Con=control; LPS =100 nmol/L LPS; L + N = LPS + NGF; L + P =LPS + PDTC. Data are expressed mean ± SD. **P* < 0.01 vs. control; #*p* < 0.05 vs. LPS injury groups.

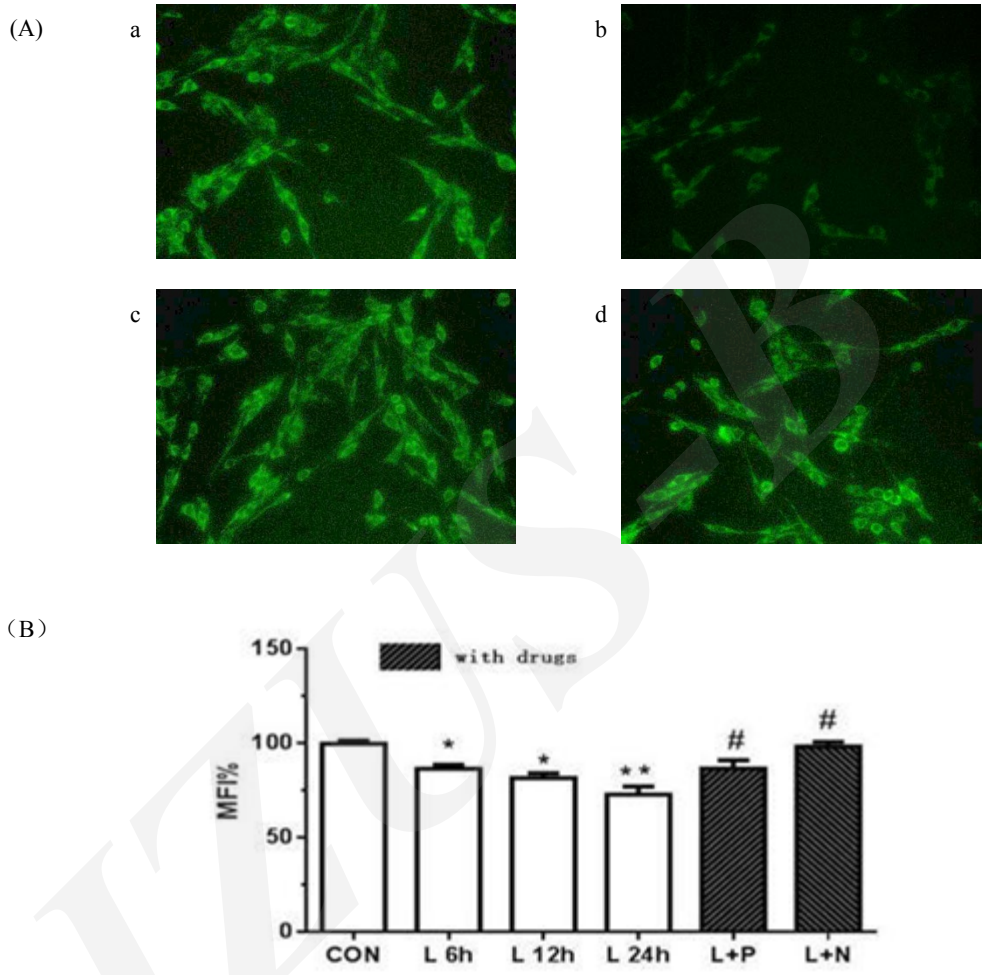



**Fig. 4 Mitochondrial transmembrane potential by Rho123 staining**

(A) PC12 cells were observed by fluorescence microscope. (a): control; (b): LPS; (c): LPS+NGF; (d): LPS+ PDTC.(B) Quantitative analysis of fluorescence intensity of MTP. Con=control; L 6h=100nmol/L LPS 6h; L 12h=100nmol/L LPS 12h; L 24h=100nmol/L LPS 24h; L+P=100nmol/L LPS+20μmol/L PDTC; L+N=100nmol/L LPS+50ng/L NGF.

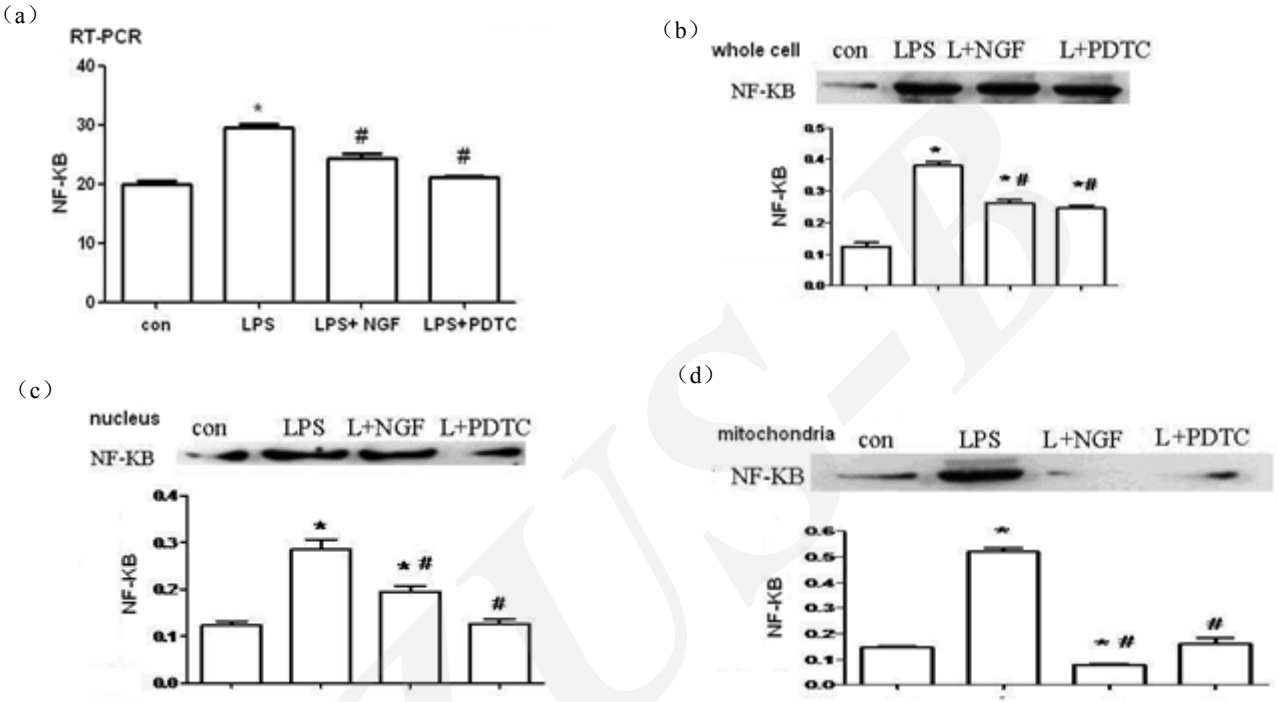



**Fig.5 The expression of NF-κB by RT-PCR and western blot.**

(a) The expression of NF-κB by RT-PCR. Con = control; LPS = LPS damage; LPS + NGF = LPS + NGF; LPS+PDTC =LPS + PDTC. (b) The expression of NF-κB by western blot in whole cell; (c) The expression of NF-κB by western blot in nucleus; (d) The expression of NF-κB by western blot in mitochondria; Data are expressed mean ± SD. **P* < 0.01 vs. control; # *p* < 0.05 vs. LPS injury groups.

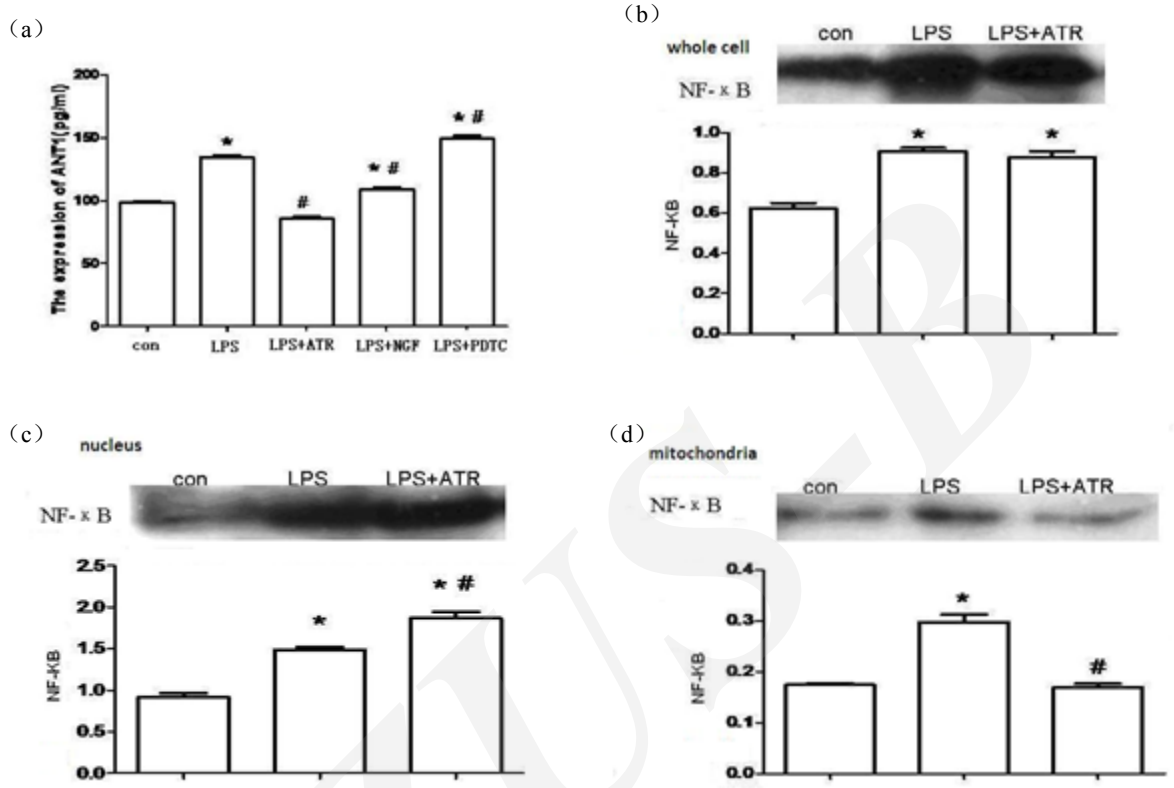



**Fig. 6 The effect of ATR and the activity of ANT1 on PC12 cells**

(a)The effect of ATR, NGF and PDTC on PC12 cells by determining the activity of ANT1 by ELISA.
(b)The effect of ATR on LPS-induced NF-κB (p65) activation in whole cells by western blot.
(c)The effect of ATR on LPS-induced NF-κB (p65) activation in the nucleus by western blot.
(d)The effect of ATR on LPS-induced NF-κB (p65) activation in the mitochondria by western blot.
Con = control; LPS = LPS damage; LPS + ATR =LPS + ATR. LPS + NGF= LPS + NGF; LPS + PDTC =LPS + PDTC
Data are expressed mean ± SD. *P<0.01 vs. control; #p<0.05 vs. LPS injury groups.