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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)**

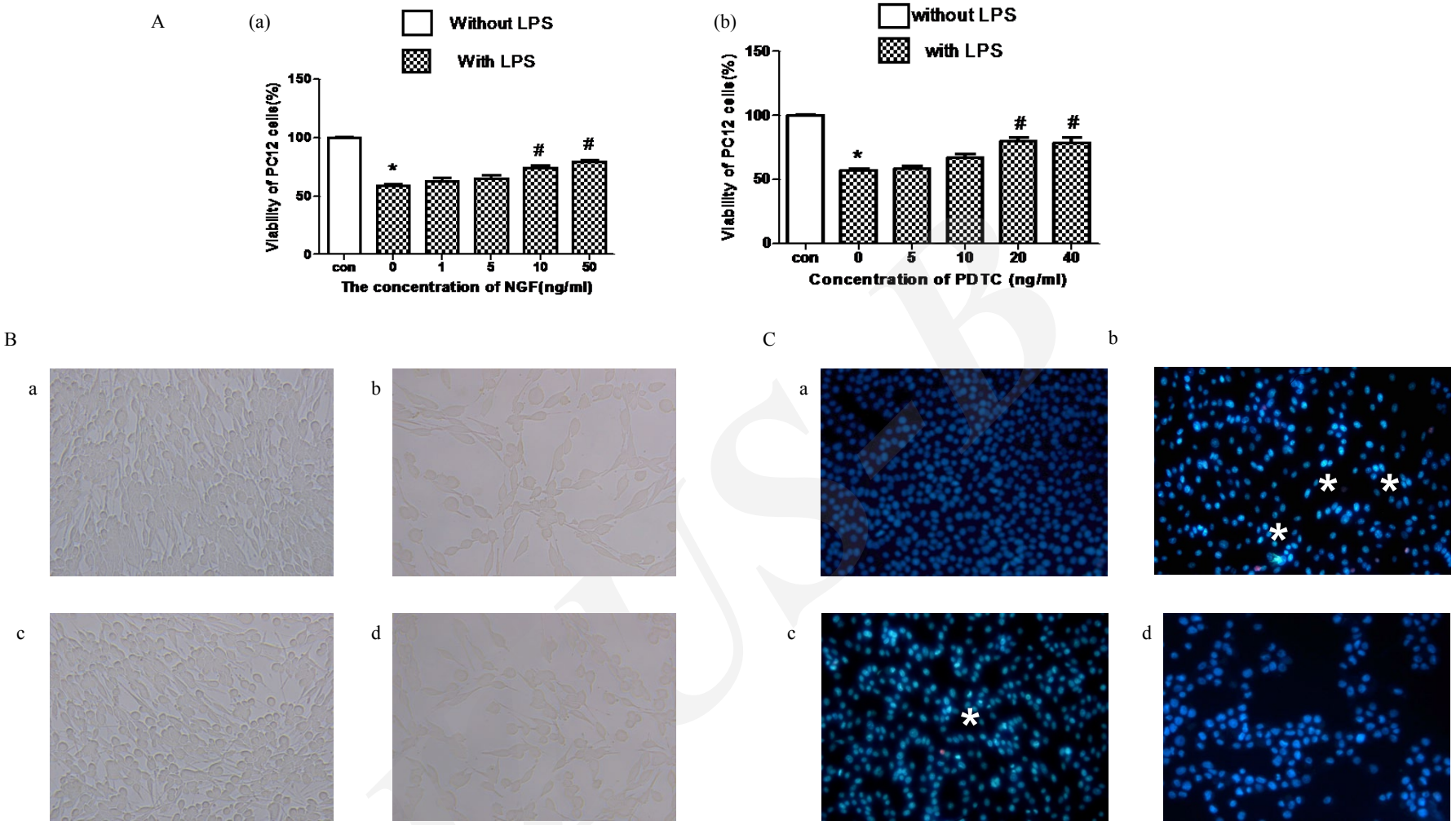

Fig. 1 The effect of nerve growth factor and PDTC on PC12 cells.

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.

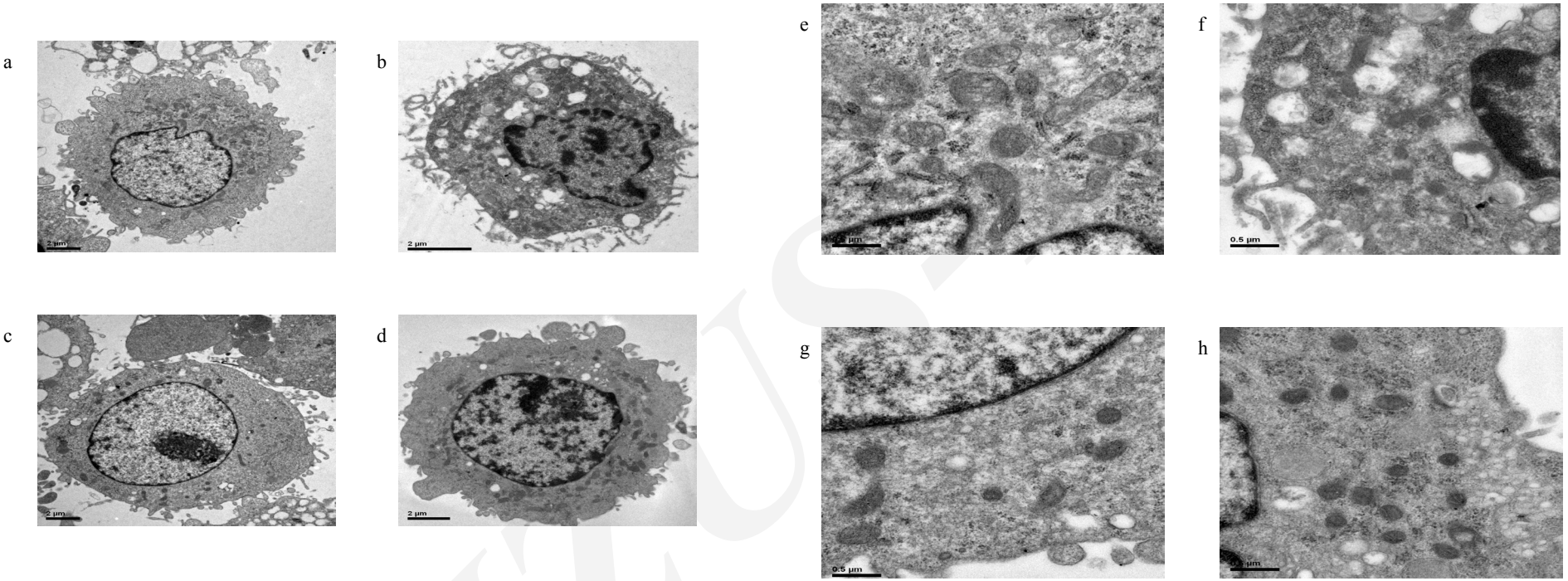

Fig. 2 Subcellular morphology observed under transmission electron microscope.

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.

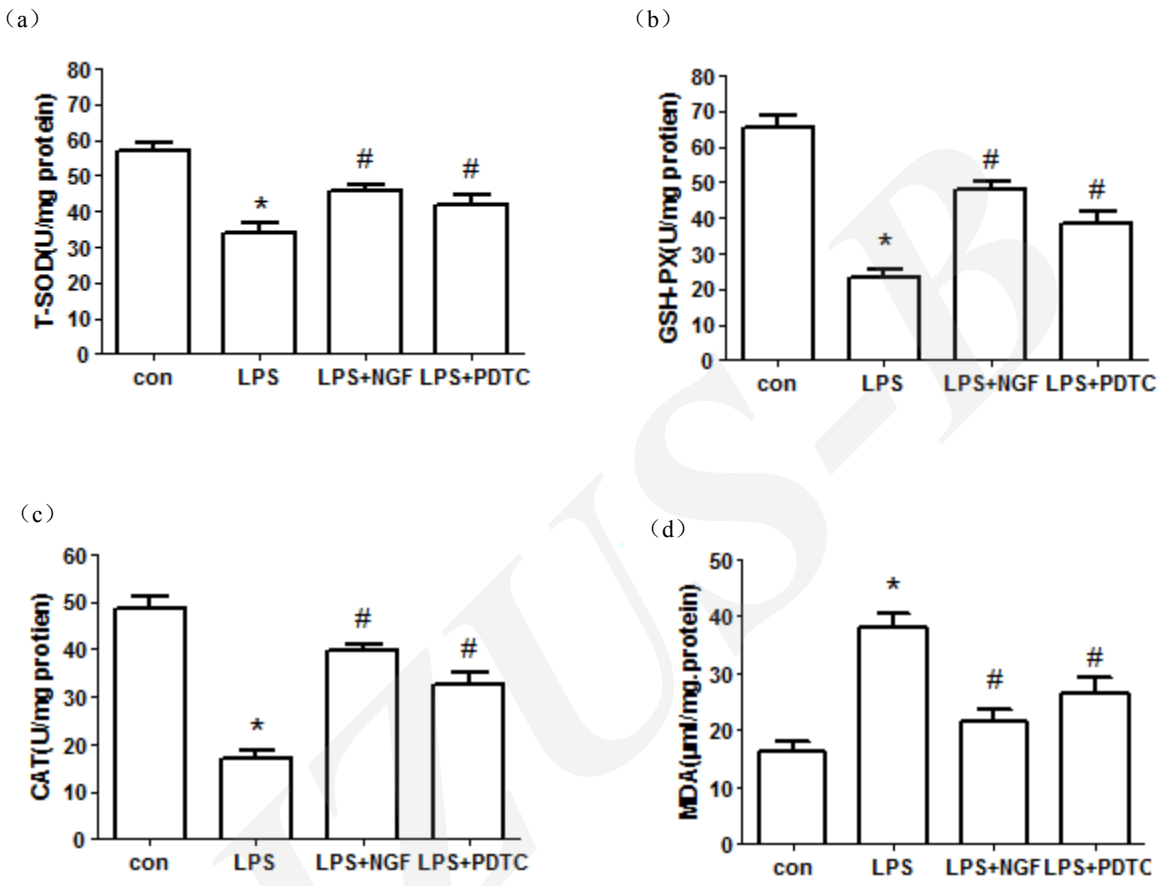

Fig. 3 The effect of NGF and PDTC on PC12 cells by determining the activity of oxidation and anti-oxidation enzyme. (a)SOD; (b) GSH-PX; (c) CAT; (d) MAD. Con=control; LPS =100 nmol/L LPS; L + N = LPS + NGF; L + P =LPS + PDTC. Data are expressed mean ± SD. **P* < 0.01 vs. control; #*p* < 0.05 vs. LPS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

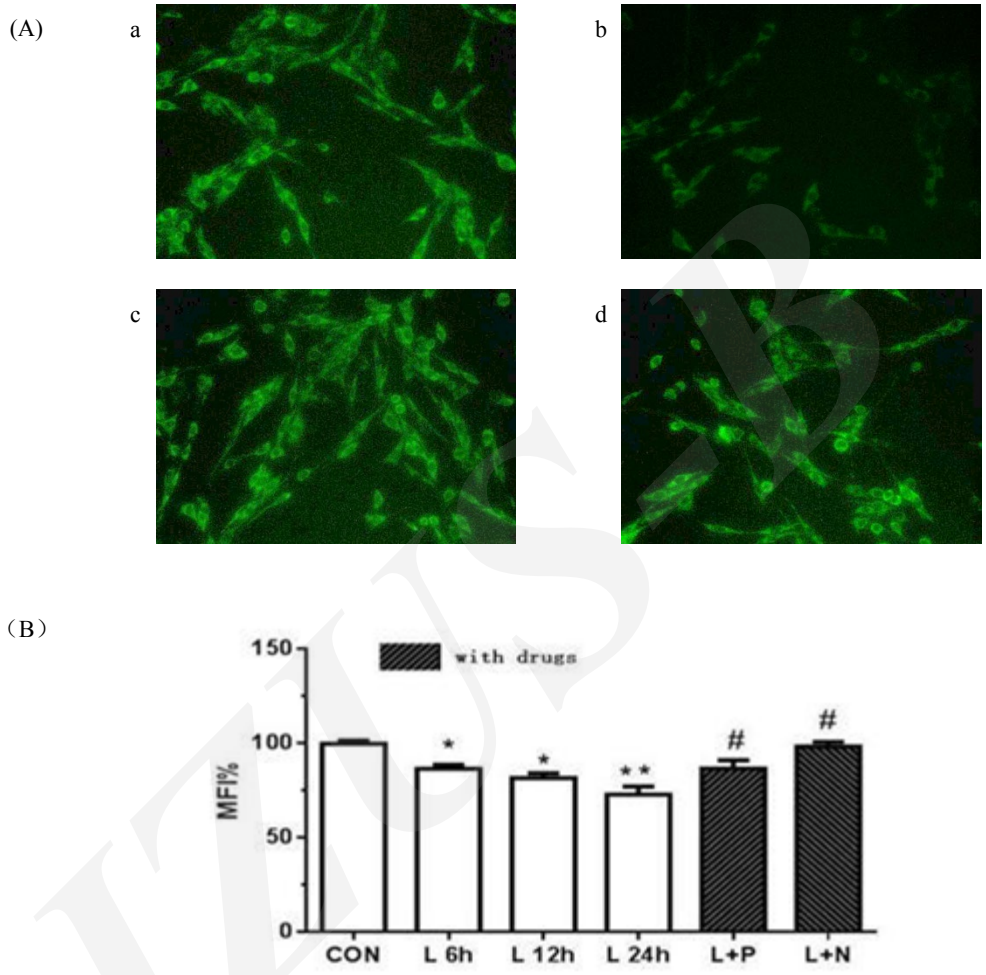

Fig. 4 Mitochondrial transmembrane potential by Rho123 staining

(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.

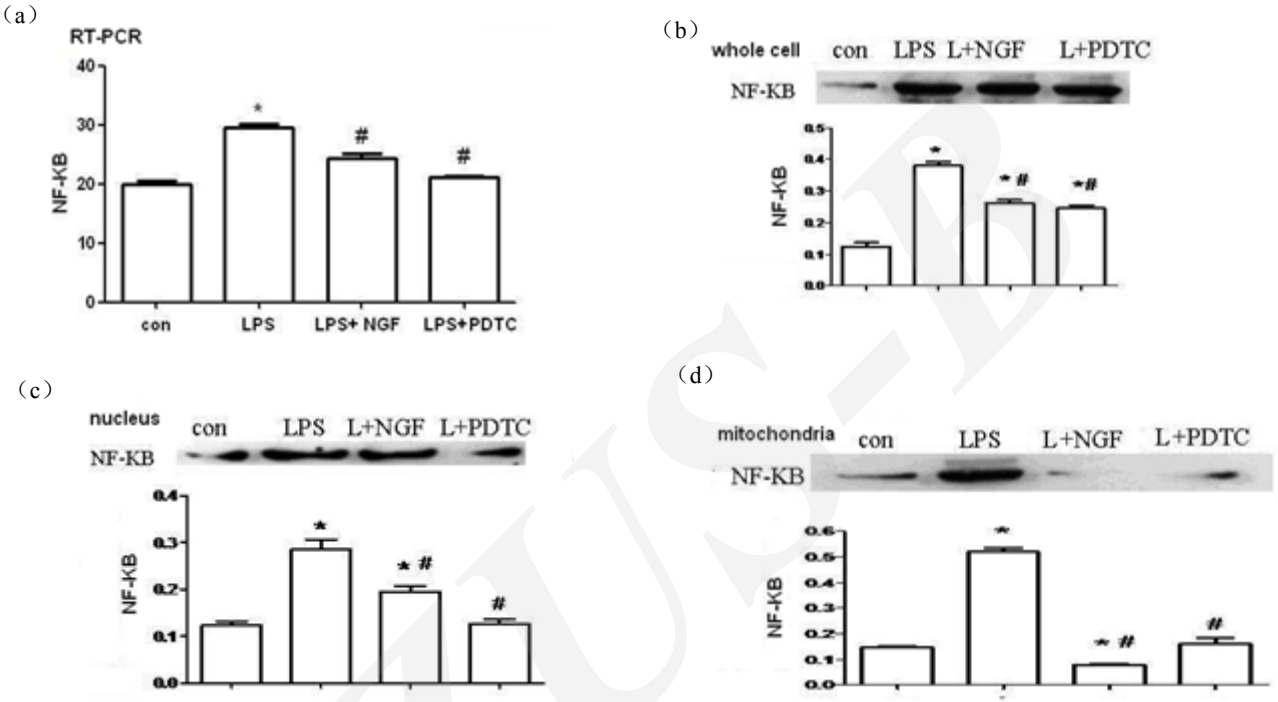

Fig.5 The expression of NF-κB by RT-PCR and western blot.

(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.

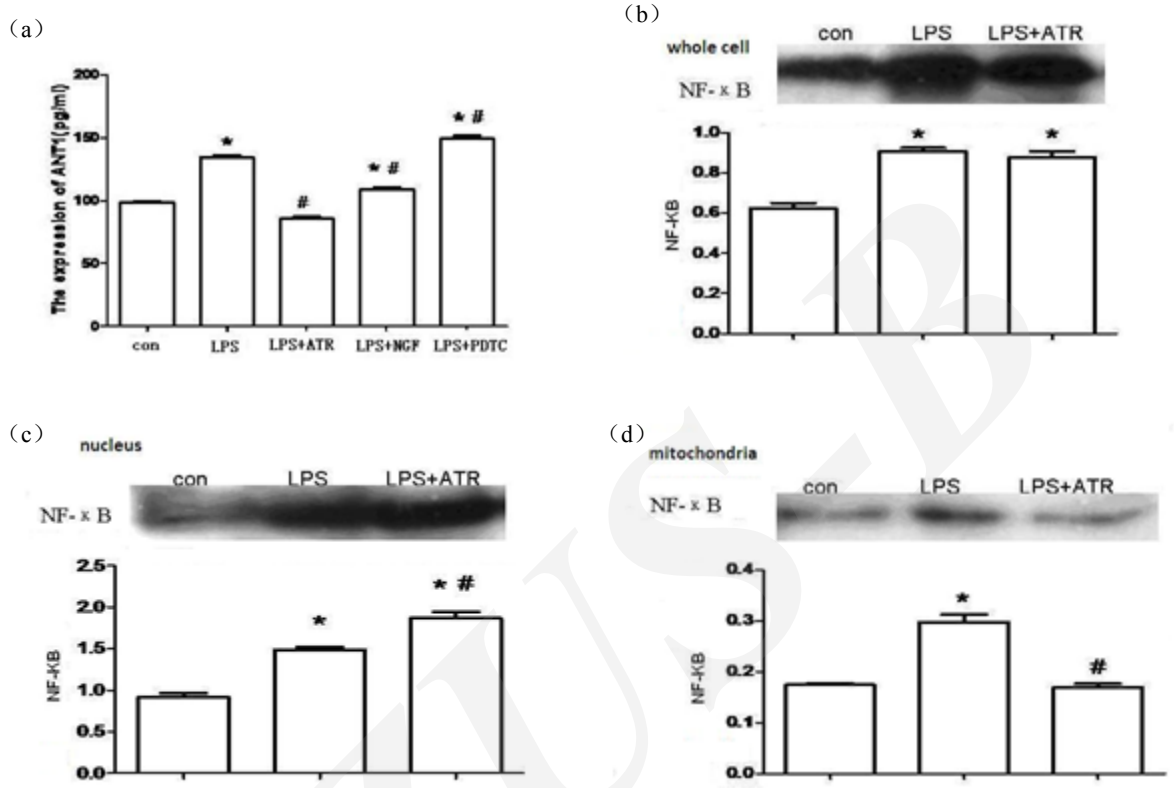

Fig. 6 The effect of ATR and the activity of ANT1 on PC12 cells

(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.