N-actylcysteine inhibits diethyl phthalate induced inflammation via JNK and STAT pathway in RAW264.7 macrophages

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NTRODUCTION

Phthalates are widespread environmental plasticizers that induce oxidative stress and inflammation in various cell types. N-acetylcysteine (NAC), a known antioxidant, has shown protective effects in multiple diseases, but its effect on diethyl phthalate (DEP)-induced toxicity in macrophages remains unclear. This study investigated NAC's anti-inflammatory mechanisms against DEP-induced damage in RAW264.7 macrophages.

METHODS

Cell Model: RAW264.7 macrophages pretreated with NAC (10 mM) for 2h, then exposed to DEP (100-300 µM)
Measurements:
Oxidative stress markers: NO, ROS, GSH/GSSG ratio
Inflammatory mediators: PGE2, IL-1β levels (ELISA)
Gene expression: IL-1β, IL-6, COX-2, iNOS (qRT-PCR)
Signaling pathways: MAPK (ERK, JNK, p38) and STAT1/3 (Western blot)

RESULTS

DEP (100-300 µM) significantly increased COX-2, iNOS protein levels and inflammatory cytokines (IL-1β, IL-6, TNF-α)
NAC pretreatment effects:
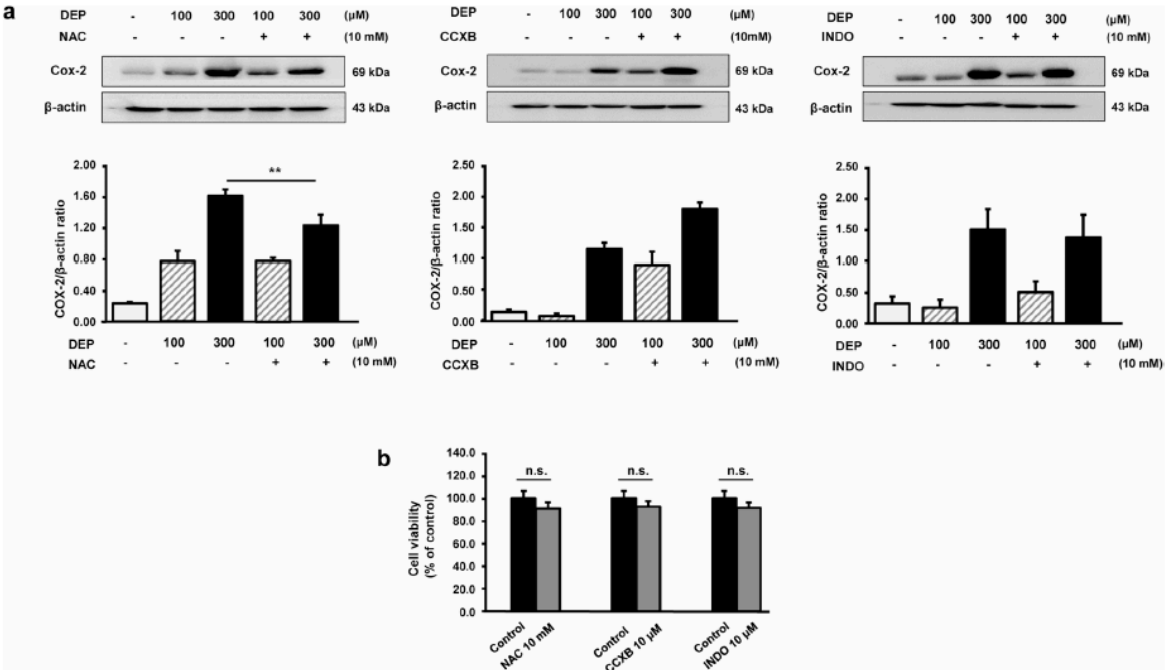↓ NO, ROS, PGE2, IL-1β levels
↑ GSH/GSSG ratio
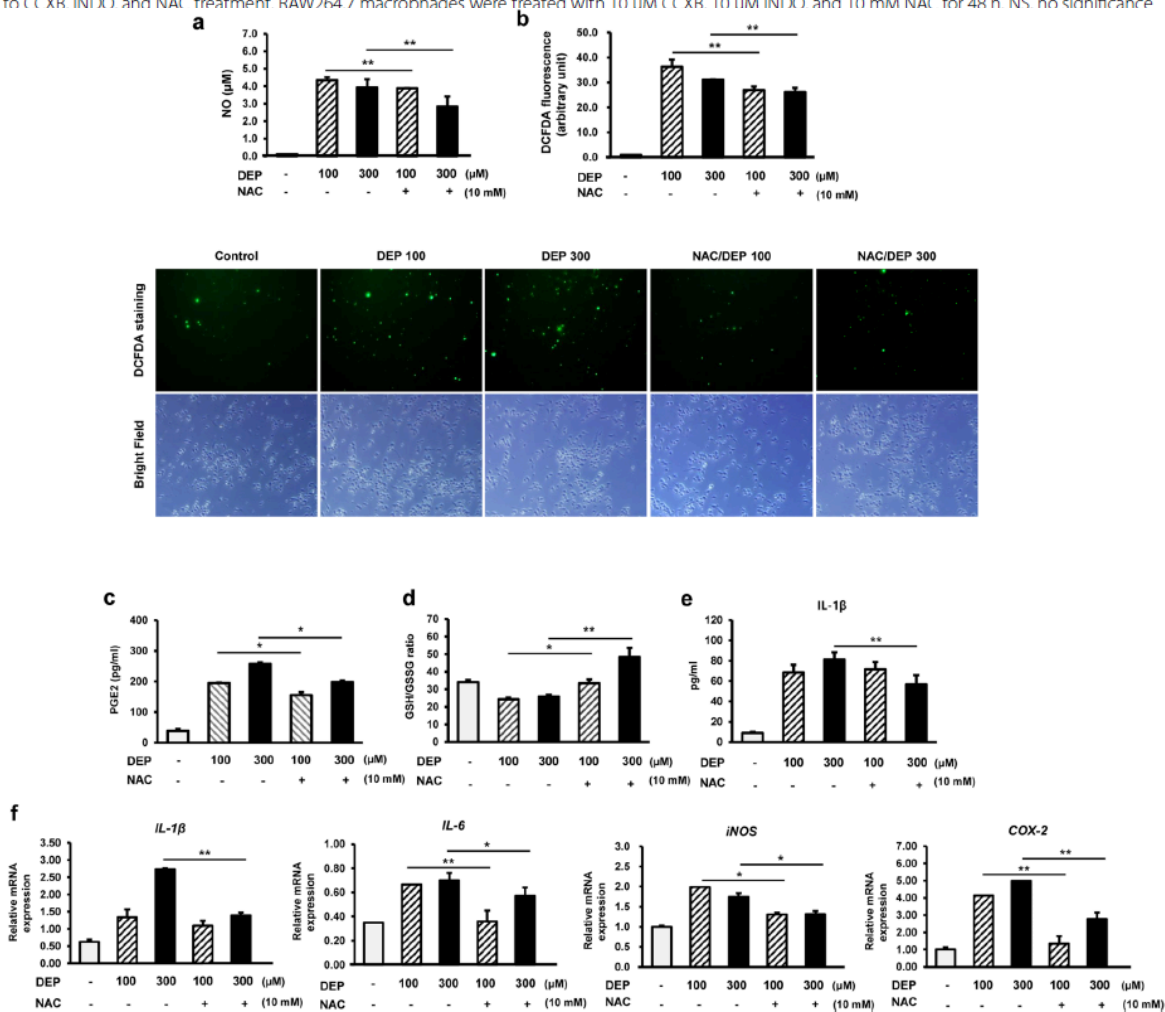↓ mRNA expression of IL-1β, IL-6, COX-2, iNOS
↓ Phosphorylation of JNK, STAT1, and STAT3
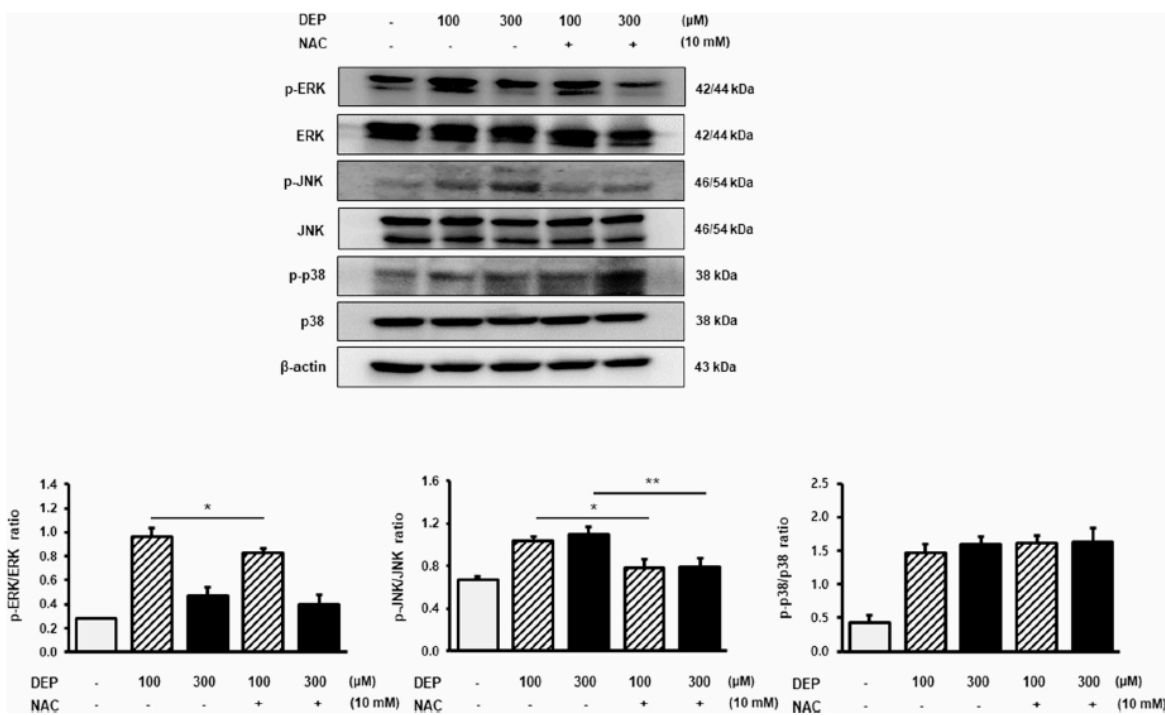
CONCLUSION

NAC effectively inhibits DEP-induced inflammation in RAW264.7 macrophages by suppressing oxidative stress and downregulating inflammatory mediators through the MAPK/JNK and STAT1/3 signaling pathways. This is the first study demonstrating NAC's protective effects against DEP toxicity in macrophages, suggesting its potential as a therapeutic agent for phthalate exposure-related inflammation.
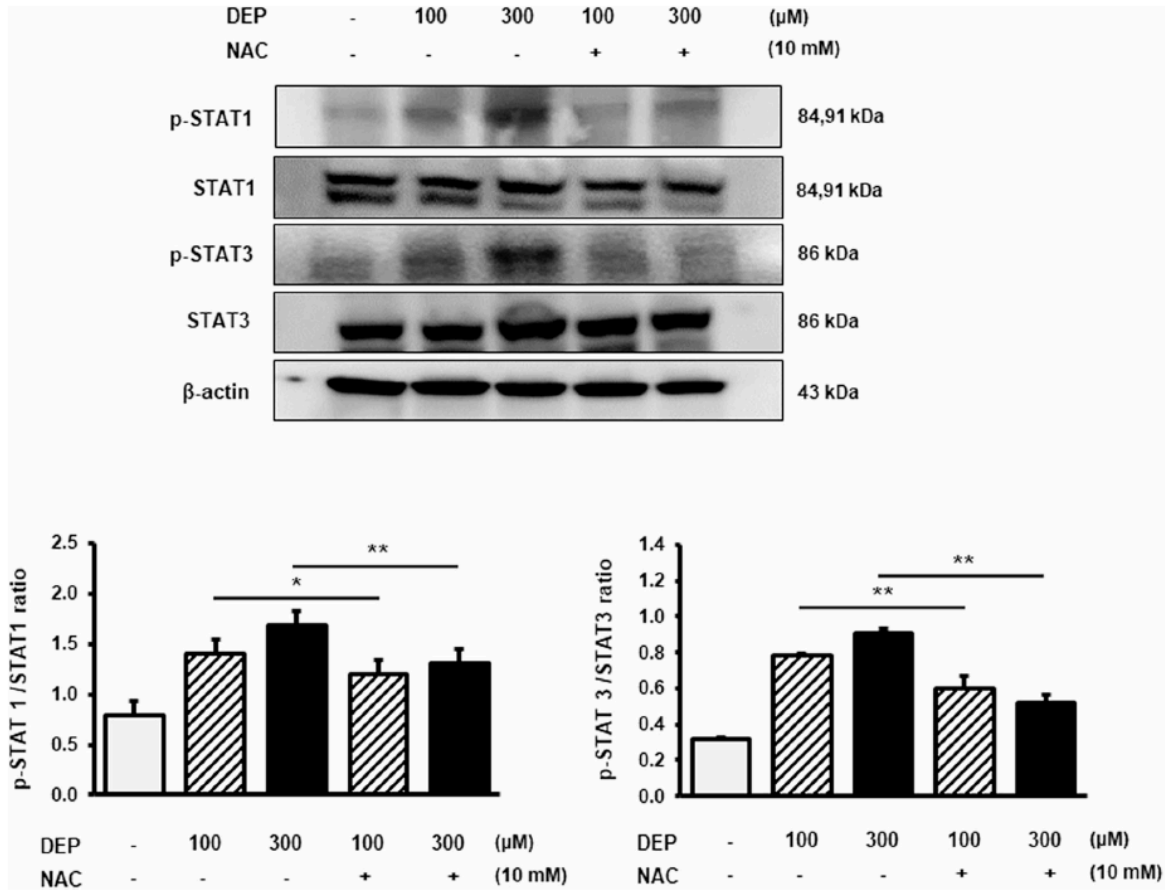

Fig. 2 NAC ameliorates DEP-induced inflammation in RAW264.7 macrophages. (a) RAW264.7 macrophages were pretreated with CCXB (COX-2 inhibitor), INDO (COX inhibitor), or NAC (antioxidant) for 2 h, followed by DEP exposure. COX-2 protein levels were determined using western blot. The expression levels of the proteins were quantified using ImageJ. β-actin was used as the internal control. Data are presented as the mean±standard deviation. *p<0.05 and **p<0.01 vs. vehicle control. Data were obtained from three independent experiments. (b) Viability of RAW264.7 macrophages exposed to CCXB, INDO, and NAC treatment. RAW264.7 macrophages were treated with 10 µM CCXB, 10 µM INDO, and 10 mM NAC for 48 h. NS, no significance
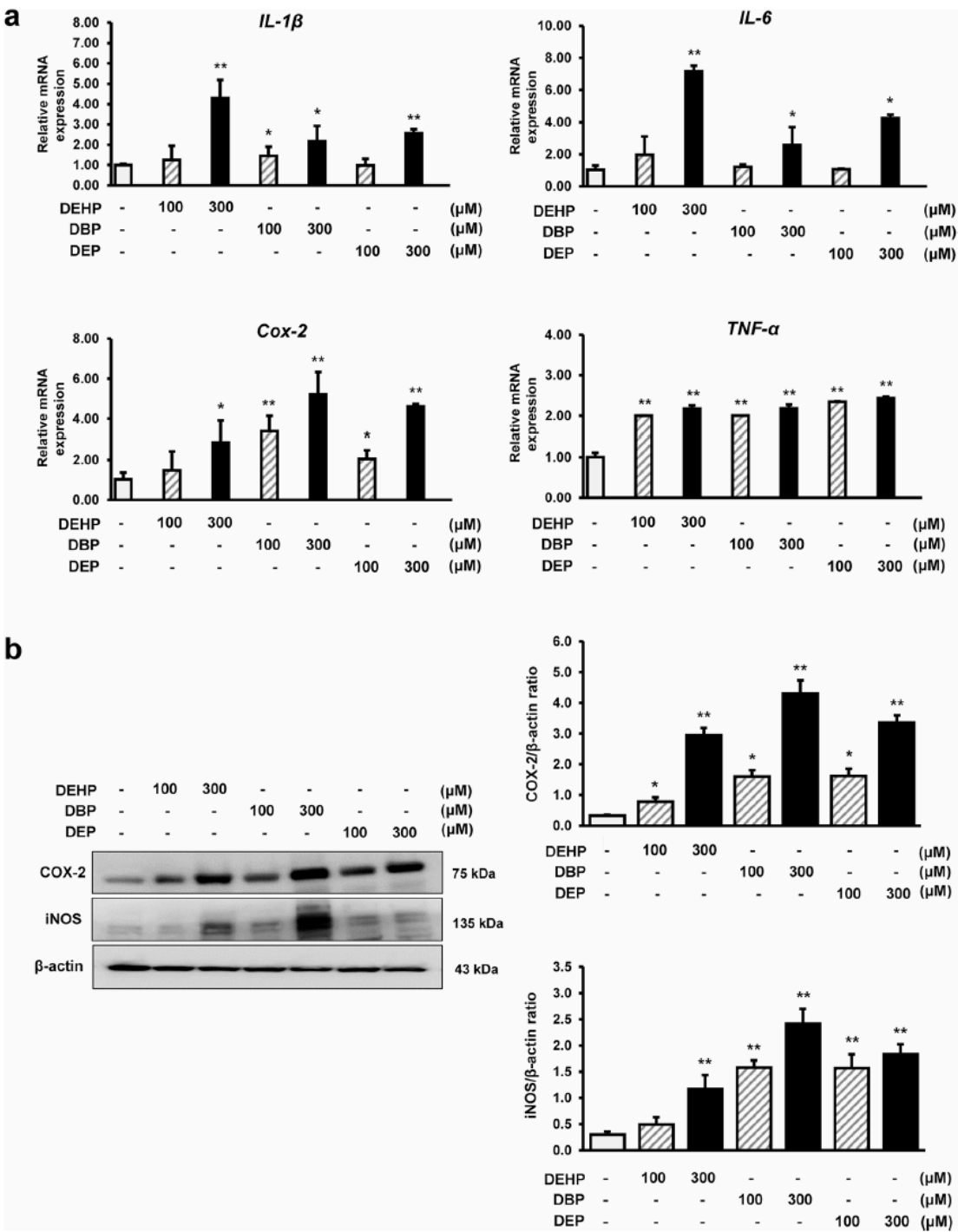

Fig. 3 NAC reduces oxidative stress and inflammation in RAW264.7 macrophages exposed to DEP. NAC pre-treatment alleviated (a) NO, (b) ROS, (c) PGE2 level, (d) GSH/SSSG ratio, (e) IL-1β level, and (f) inflammatory-related gene expression, including IL-1β, IL-6, TNF-α, and COX-2, compared with those of DEP-induced RAW264.7 macrophages. Data are presented as the mean±standard deviation. *p<0.05 and **p<0.01 vs. vehicle control. Data were obtained from three independent experiments

Fig. 4 Effect of NAC on MAPK pathway proteins in DEP-induced RAW264.7 macrophages. Western blotting analysis was performed to determine the expression of phosphorylated ERK, JNK, and pp38 protein levels in RAW264.7 macrophages treated with NAC following DEP-induced inflammation. The expression levels of the proteins were quantified using ImageJ. β-actin served as the loading control. Data are presented as the mean±standard deviation. *p<0.05 and **p<0.01 vs. vehicle control. Data were obtained from three independent experiments

Fig. 5 Effect of NAC on expression of the STAT1/3 pathway in DEP-induced RAW264.7 macrophages. Western blotting analysis was performed to determine the expression of phosphorylated STAT1 and STAT3 protein levels in RAW264.7 macrophages treated with NAC following DEP-induced inflammation. The expression levels of the proteins were quantified using ImageJ. β-actin served as the loading control. Data are presented as the mean±standard deviation. *p<0.05 and **p<0.01 vs. vehicle control. Data were obtained from three independent experiments

Fig. 1 Inflammatory gene expression in RAW264.7 macrophages exposed to DEHP, DBP, and DEP. (a) mRNA expression of IL-1β, IL-6, TNF-α, and COX-2 in RAW264.7 macrophages exposed to 0, 100, or 300 µM of phthalate for 24–48 h, using qRT–PCR. Data are presented as the mean±standard deviation. *p<0.05 and **p<0.01 vs. vehicle control. Data were obtained from three independent experiments. (b) Protein levels of COX-2 and iNOS in RAW264.7 macrophages following treatment with 0, 100, or 300 µM of phthalates for 24–48 h, using western blot analysis. The expression levels of the proteins were quantified using ImageJ. β-actin was used as the internal control. Data are presented as the mean±standard deviation. *p<0.05 and **p<0.01 vs. vehicle control. Data were obtained from three independent experiments