Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Autophagy-mediated Clearance of Ubiquitinated Mutant Huntingtin by Graphene Oxide

Peipei Jin[a#], Pengfei Wei[a], Yunjiao Zhang[a], Jun Lin[a], Rui Sha[a], Yi Hu[a], Jiqian Zhang[b], Wei Zhou[a],Han Yao[a], Li Ren[c]*, James Y. Yang[d], Yanchun Liu[e] and Longping Wen[a]**

[a]The CAS Key Laboratory of Innate Immunity and Chronic Disease, Innovation Center for Cell Signaling Network, School of Life Sciences and Medical Center, University of Science and Technology of China, Hefei, Anhui 230027, China.

[b]Department of Anesthesiology, First Affiliated Hospital, Anhui Medical University, Anhui, China

[c]School of Materials Science and Engineering, South China University of Technology , Guangzhou 510641 , P.R. China.

[d]School of Life Science, Xiamen University, Xiamen, China

[e]The Key Laboratory of Energy-Efficient Functional Ceramics and Applied Technology of Guangdong Province, Guangzhou Redsun Gas Applications Co., LTD, Guangzhou 510435, China.

[#] Present address: School of Materials Science and Engineering, South China University of Technology, Guangzhou 510641, P.R. China.

***Corresponding author:**

yunjiaoz@ustc.edu.cn, psliren@scut.edu.cn or lpwen@ustc.edu.cn

Supplementary Data

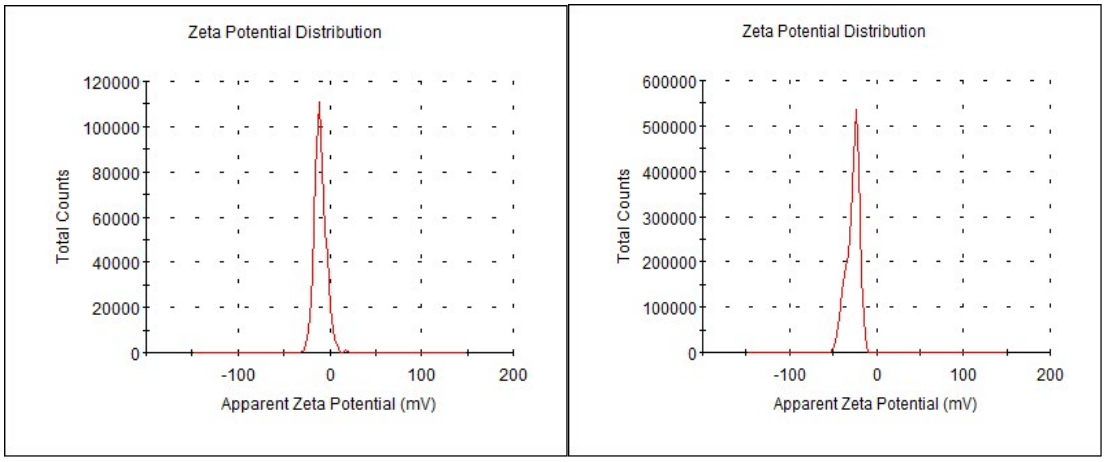


Figure S1. Zeta potential distribution of graphene oxide. Zeta potential distribution of large size GO (A) and small size GO (B) were measured by dynamic light scattering.

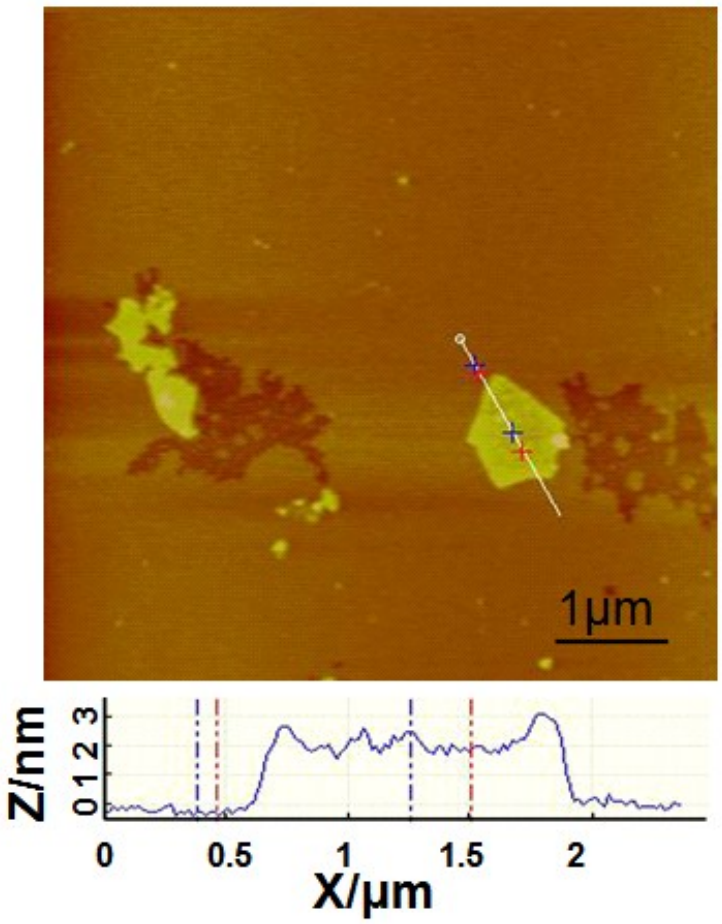
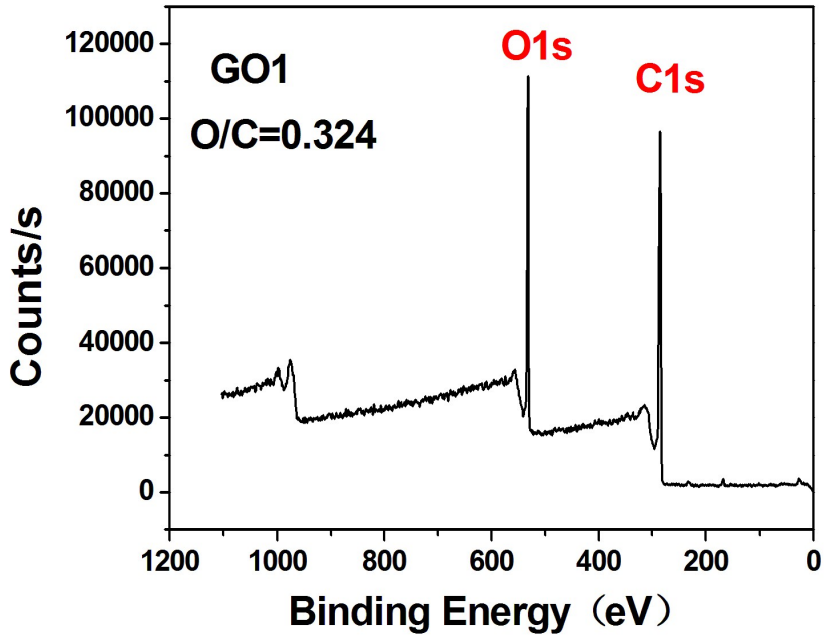
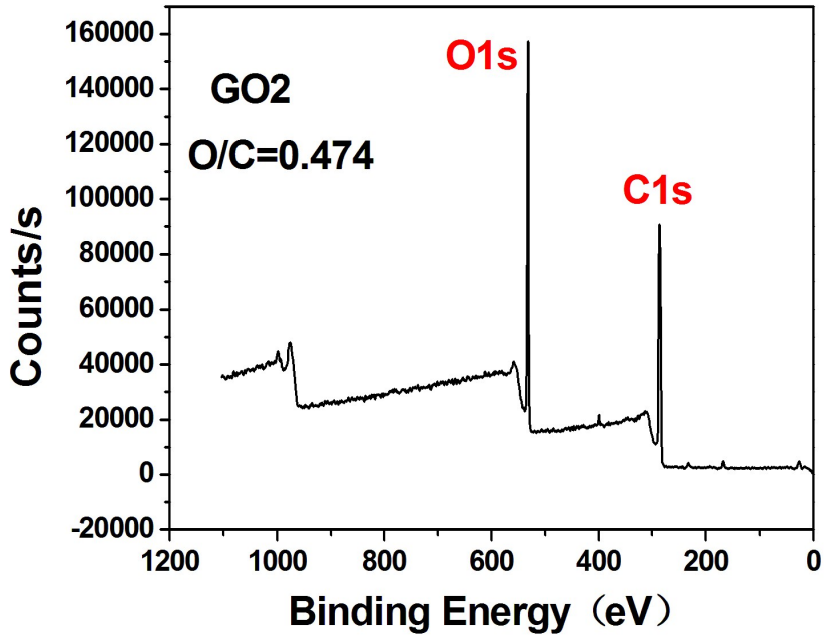


Figure S2. AFM of GO-M. The morphology and thickness of graphene oxide with multilayer were measured by AFM.


GO1
O/C=0.324
O1s
C1s
Counts/s
120000
100000
80000
60000
40000
20000
0
1200
1000
800
600
400
200
0
Binding Energy（eV）


GO2
O/C=0.474
O1s
C1s
Counts/s
160000
140000
120000
100000
80000
60000
40000
20000
0
-20000
1200
1000
800
600
400
200
0
Binding Energy（eV）

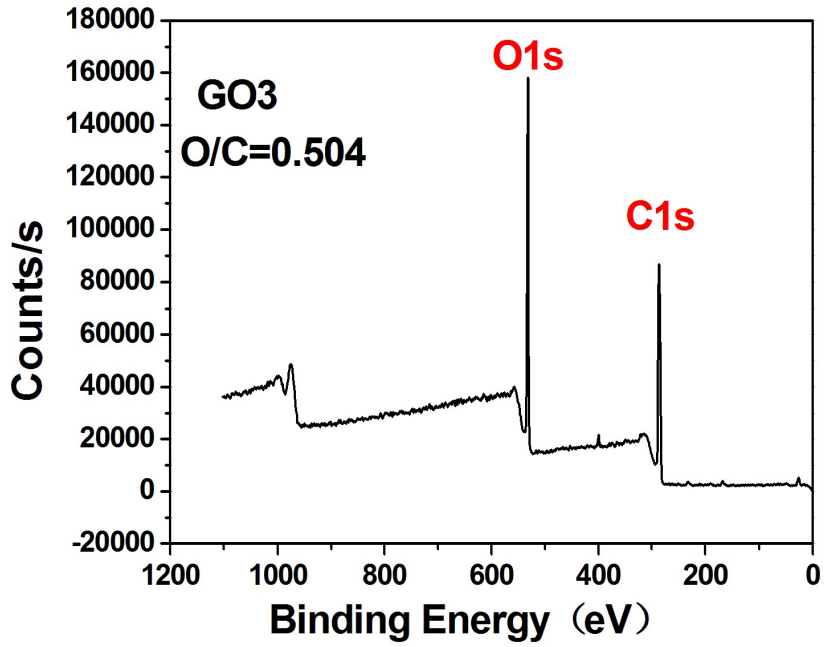


Figure S3. XPS spectra of graphene oxide. The oxidation extent of GO with different C/O ratios were measured by XPS spectra.

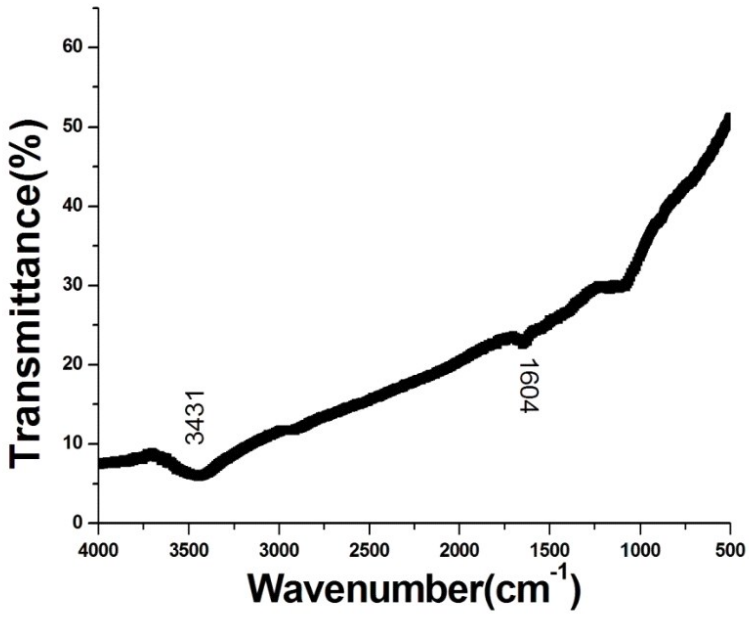


Figure S4. FTIR spectra of graphene. The surface state of graphene was characterized by FTIR spectra.

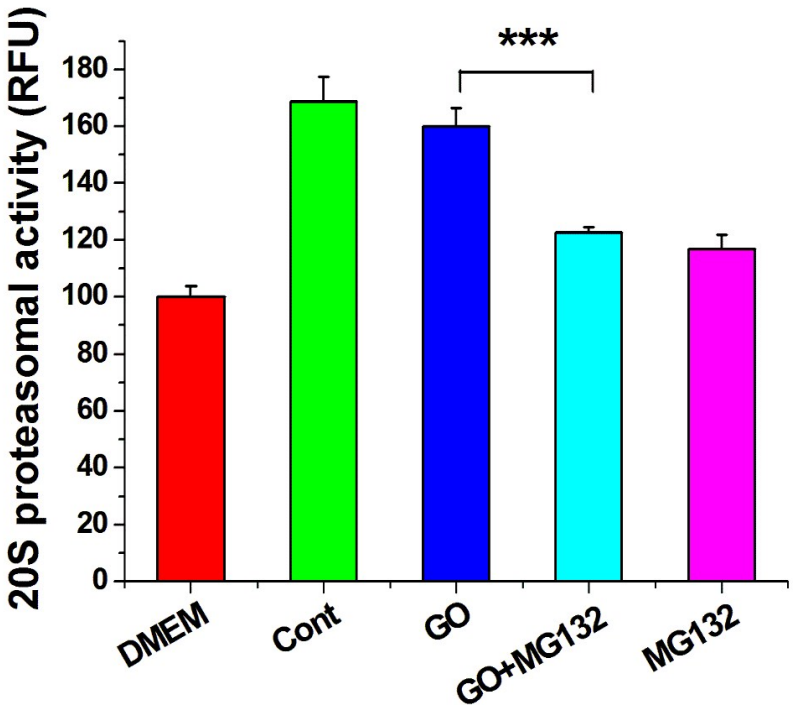


Figure S5. 20s proteasomal activity assay. The proteasome activity was detected by Fluorimetric Proteasome 20S Activity Assay Kit in GFP-Htt(Q74)/PC12 cells treated with PBS (control) or 60 μg/mL GO for 24 h in the presence or absence of MG132 (1 μM).

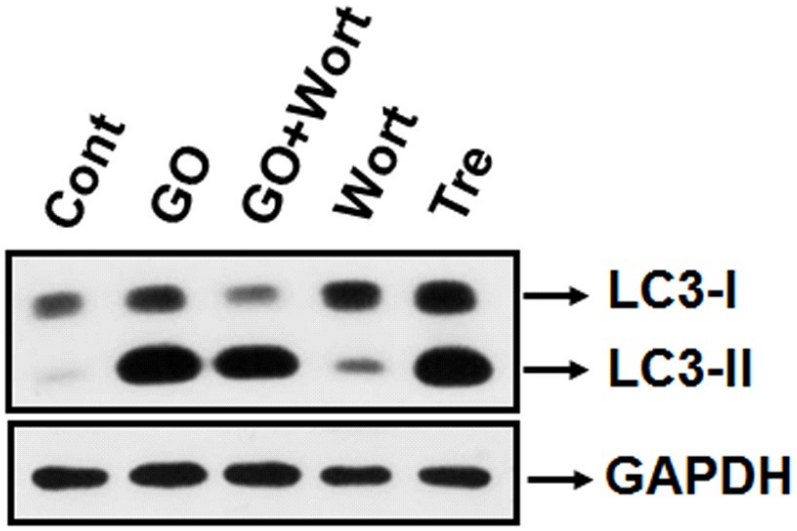


Figure S6. Wortmannin reduced the LC3 conversion induced by GO in HeLa cells. Western blotting of LC3 in HeLa cells treated with PBS (control) or 60 μg/mL GO for 24 h in the presence or absence of 3-MA (2.5 mM).

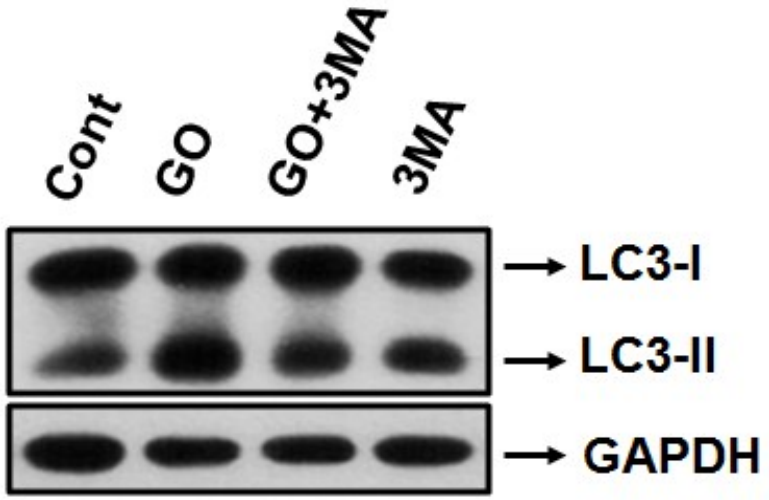


Figure S7. 3-MA reduced the LC3 conversion induced by GO in HeLa cells. Western blotting of LC3 in HeLa cells treated with PBS (control) or 60 μg/mL GO for 24 h in the presence or absence of 3-MA (2.5 mM).

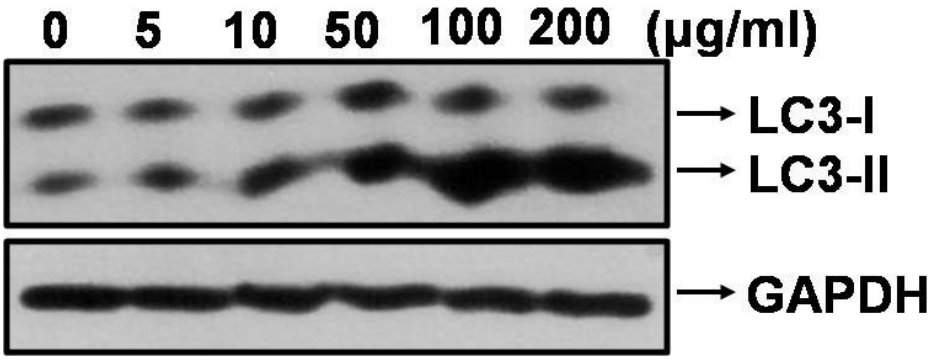


Figure S8. GO induced LC3 conversion in a dose-dependent way in HeLa cells. HeLa cells were treated with various doses of GO for 24 h and then subjected to western blotting with anti-LC3 antibody or with GAPDH as a loading control.

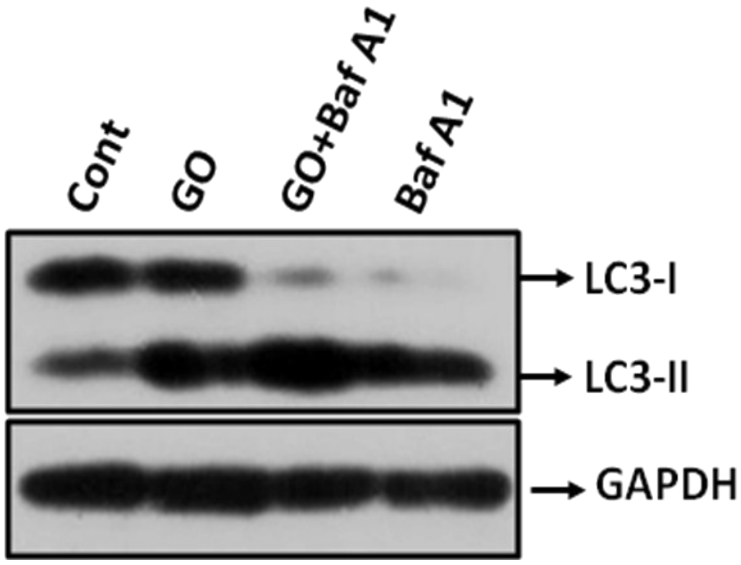


Figure S9. GO induced normal autophagic flux in HeLa cells. HeLa cells were treated with 60 μg/mL GO for 24 h in the presence or absence of Bafilomycin A1 (Baf A1, 400 nM). Endogenous LC3-II was detected by western blotting with anti-LC3 antibodies.

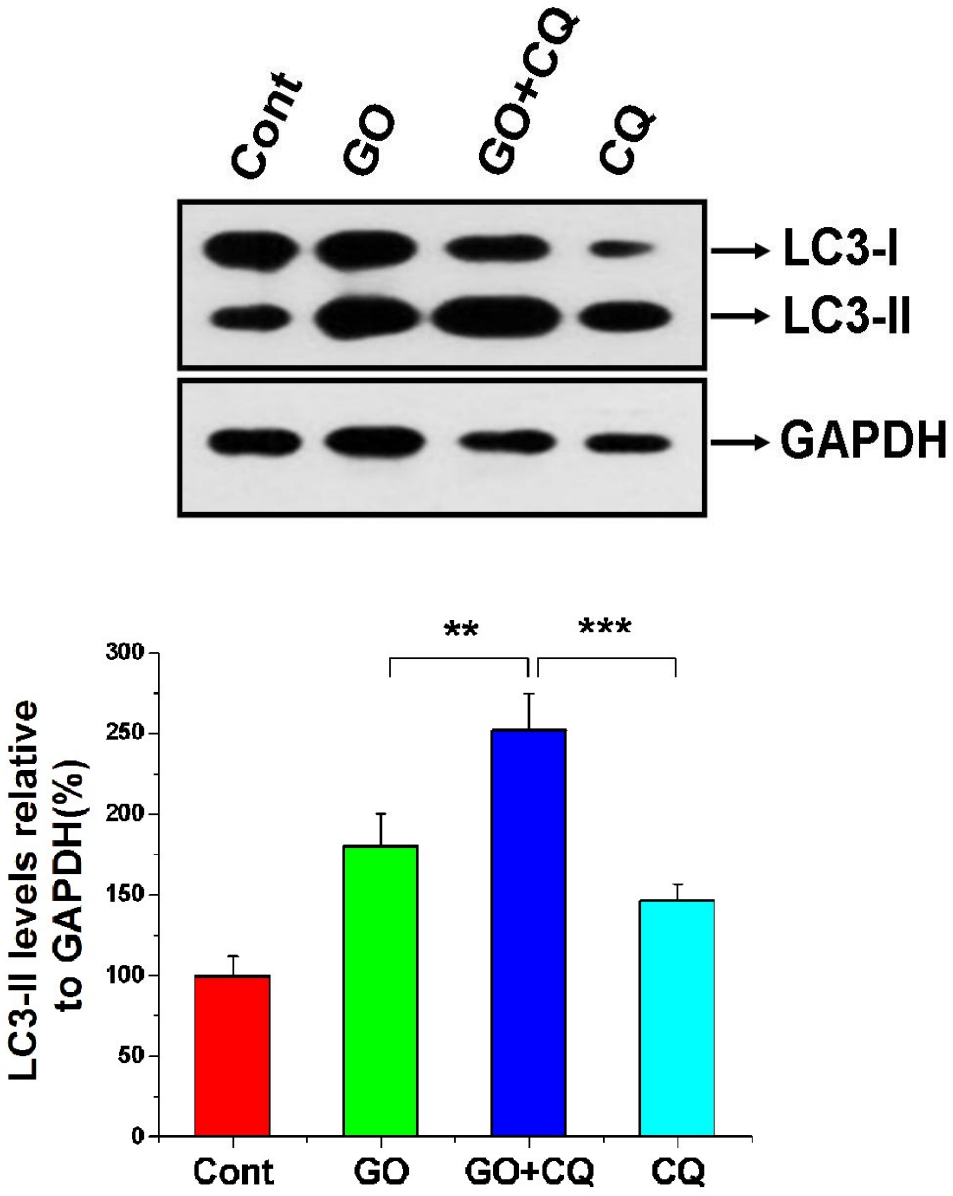


Figure S10. GO induced normal autophagic flux in HeLa cells. HeLa cells were treated with 60 μg/mL GO for 24 h in the presence or absence of chloroquine (CQ, 50 μM). Endogenous LC3-II was detected by western blotting with anti-LC3 antibodies and quantified by densitometric analysis relative to GAPDH (right panels). Mean ± SEM, n=5. **P < 0.01 ***P < 0.001 comparing to control group.