

**Title:** The neuro-toxicity of gossypol is possibly attributable to its trigger fluxes of  $\text{Na}^+$  and  $\text{K}^+$  across the plasmalemma in N2A cells

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**Aim of investigation:** Gossypol is a polyphenolic dialdehyde compound isolated from cotton seed, and has recently been proposed to be an anticancer agent. However, it may have toxicity in other organ systems. There is very little known about whether there is any toxic effect of gossypol on the nervous system. The aim of this study is to examine the effects of gossypol on  $\text{Ca}^{2+}$  level and ion movements in a neuroblastoma cell line N2A.

**Methods:** Neuroblastoma N2A cells were obtained from the American Type Culture Collection (Manassas, VA). Mitochondria membrane potential (MMP) measured by MMP Assay Kit... Total RNA was extracted from cells using a TRIzol kit (MDBio, Inc., Taipei, Taiwan). The reverse transcription reaction was performed using 2 mg of total RNA that was reverse transcribed into cDNA. Electrophysiological experiment. The  $p < 0.05$  were considered significant (ANOVA).

**Results:** Addition of gossypol induced large ion influx and efflux. After the addition of gossypol, intracellular  $\text{Ca}^{2+}$  level was elevated; however, gossypol failed to induce a  $\text{Ca}^{2+}$  signal in the absence of extracellular  $\text{Ca}^{2+}$ . When compared to the control (where there was a small amount of leaky  $\text{Ni}^{2+}$  entry), gossypol did not cause influx of  $\text{Ni}^{2+}$ . These data suggest that gossypol opens a pathway in the plasma membrane which was permeable to  $\text{Ca}^{2+}$  but not  $\text{Ni}^{2+}$ . Using an electrophysiological approach (patch clamp), shown that inward currents were triggered by -120 mV hyperpolarizing pulses; addition of gossypol triggered an increase in inward currents.

**Conclusions:** We demonstrate for the first time that in neuroblastoma N2A cells gossypol (10  $\mu\text{M}$ ) caused influx of  $\text{Ca}^{2+}$  and  $\text{Na}^+$  and evidence has also shown that gossypol could also trigger the permeation of  $\text{K}^+$ . Interestingly, the tentative pore opened by gossypol did not allow  $\text{Ni}^{2+}$  to pass. Our data therefore suggest that the pore seems to have some selectivity.

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