Evaluation of prevention of DNA damage and induction of DNA repair in *Saccharomyces cerevisiae* by *Ginkgo biloba* leaf extracts

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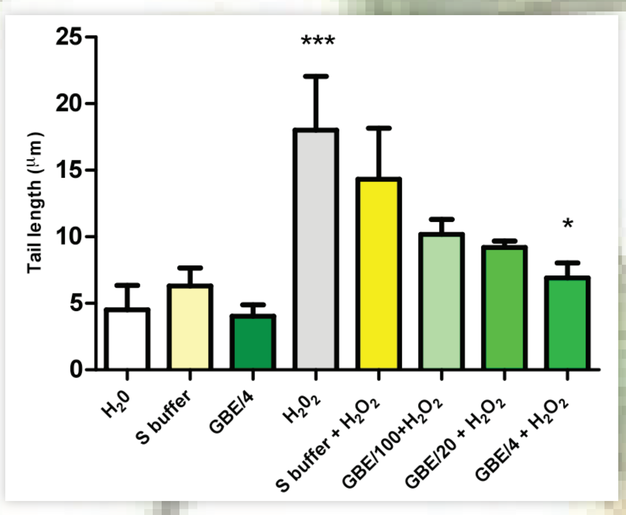
Preparation of the *G. biloba* leaf extract (GBE)

Extracts were prepared as previously reported by Ding and co-workers (Ding *et al.* 2004. *Anal Chem* 76:4332-6)

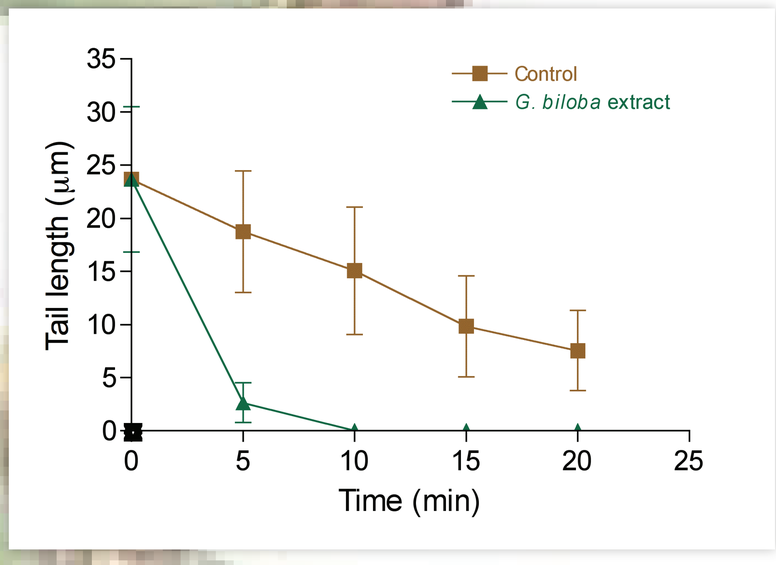
- Leafs from a local specimen were collected in Autumn
- Leafs were washed with deionized water and cut to exclude petioles
- Leafs were pulverized with a pestle into a fine powder
- 5g of the powder was extracted with 30mL of boiling deionized water and maintained at 100ºC for 5min
- The mixture was centrifuged at 200*g*, 15min and the supernatant was collected
- The extraction was repeated once and the supernatants were pooled
- Supernatants were cleared by filtration with 0.5µm and 0.2µm filters
- pH was adjusted to 6.5 with NaOH
- Extract was stored in aliquots at -20ºC

Co-incubation with GBE and H_2O_2 protects yeast cells from H_2O_2-induced DNA damage



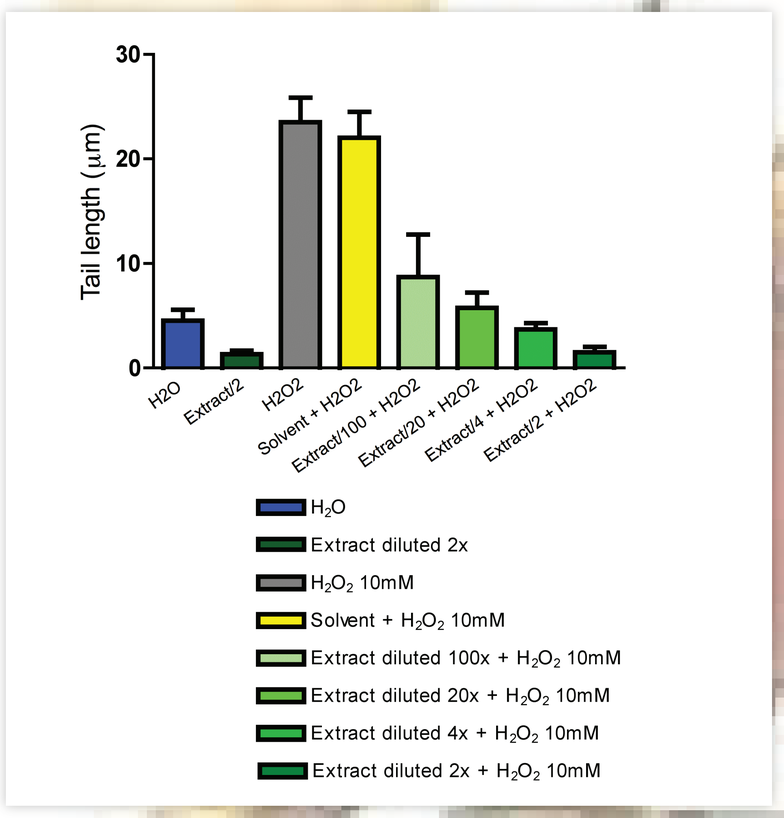
Yeast spheroplasts (BY4741 strain) were incubated with GBE (diluted 2, 4, 20 or 100 fold) and 5mM H_2O_2 for 20min. Spheroplasts were washed and analyzed by the comet assay.

GBE improves repair kinetics in H_2O_2-induced DNA damage



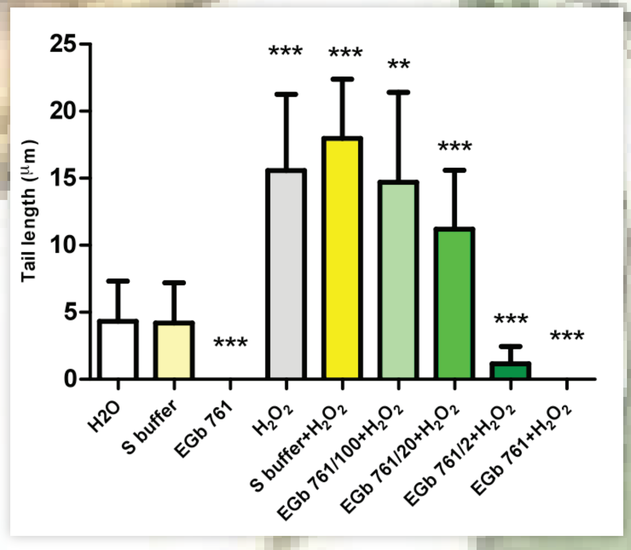
Yeast spheroplasts (BY4741 strain) were incubated with GBE for 20min, washed, and subsequently incubated with H_2O_2 5mM for 20min. After washing, spheroplasts were allowed to recover DNA damage at 30ºC. At each specified time point, an aliquot of the spheroplasts was analyzed by the comet assay.

Pre-incubation with GBE protects yeast cells from H_2O_2-induced DNA damage

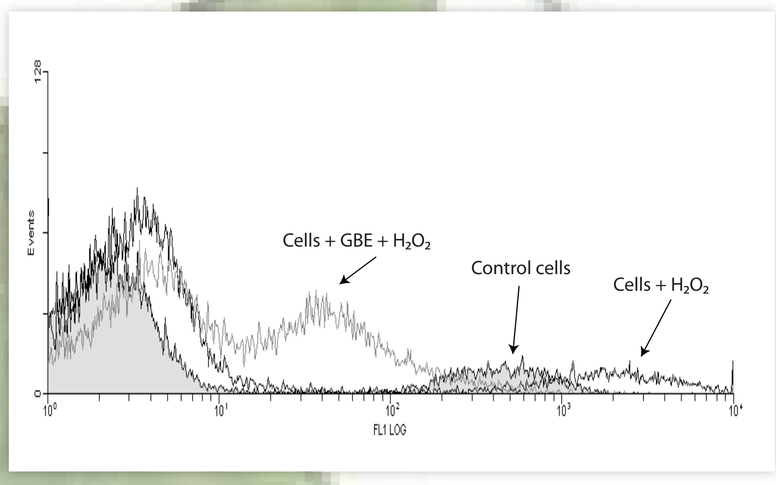


Yeast spheroplasts (BY4741 strain) were incubated with GBE (diluted 2, 4, 20 or 100 fold) for 20min, washed, and subsequently incubated with 5mM H_2O_2 for 20min. Spheroplasts were washed and analyzed by the comet assay.

The standardized GBE EGb761 yielded similar DNA protection as our extract

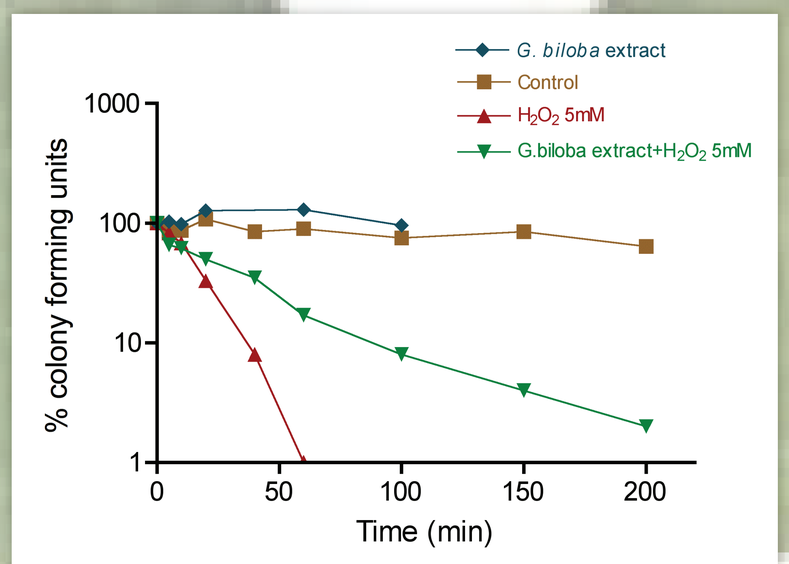


GBE protects yeast cells against oxidative stress

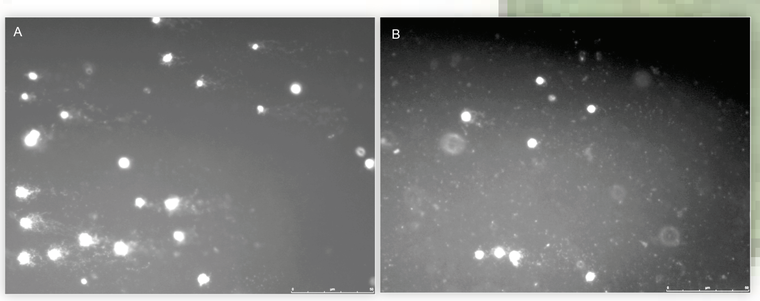


Filled black line: cells loaded with dichlorofluorescein diacetate (H_2DCFDA) after incubation for 60min in the dark.
Empty black line: cells loaded with H_2DCFDA and incubated with 10mM H_2O_2 for 20min.
Empty grey line: cells loaded with H_2DCFDA, incubated with GBE diluted 2x for 20min and subsequently incubated with 10mM H_2O_2 for 20min.

GBE protects yeast cells from H_2O_2-induced loss of viability

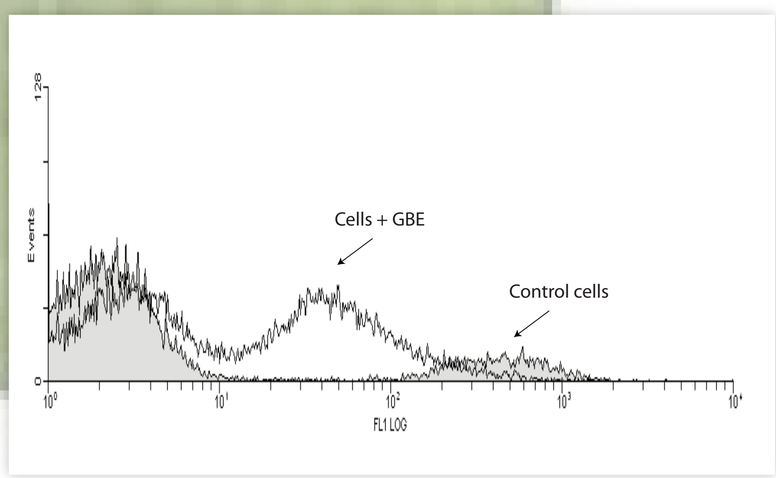


Yeast cells (BY4741 strain) were incubated with *G. biloba* leaf extract for 20min, washed, and subsequently incubated with H_2O_2 5mM. Aliquots were removed at each specified incubation time and plated on plates containing rich medium.



Comets from yeast cells treated with buffer (A) or GBE diluted 2x (B) before incubation with 5mM H_2O_2.

GBE decreases intracellular oxidation in the absence of exogenous oxidative stress



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