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Potential of phenolic extracts from Brewer's spent grain to protect against oxidant-induced DNA damage

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Brewers' spent grain (BSG), the residual solid fraction of barley malt remaining after the production of wort, contains relatively high amounts of phenolic acids including ferulic acid, p-coumaric acid, sinapic acid and caffeic acid[(1)]. BSG has the potential to be utilised as a valuable source of antioxidant, anti-inflammatory and anti-carcinogenic compounds[(2)]. The objective of the study was to establish the *in vitro* antioxidant effect of these extracts using U937, human leukaemic cell line.

Four extracts were prepared from both pale (P1–P4) and dark (B1–B4) BSG. Extract 1 was an aqueous extract of BSG and contained free phenolics. Extract 2 was extracted from BSG sediment, following protein extraction, using 1 M NaOH which releases bound phenolics. Extract 3 was an aqueous wash of the sediment used for extract 2 and contained the remaining bound phenolics. Extract 4 was the supernatant from a 110 mM NaOH protein extract of BSG and contains the phenolics that are extracted at this NaOH concentration. The total phenol content of each of the extracts was measured. U937 cells were treated with increasing concentrations of the phenolic extracts for 24 h and the MTT assay was used to determine cell viability. DNA damage in U937 cells was evaluated by the comet assay, following pre-treatment of the cells with 2.5% (v/v) BSG extracts or 1 μg/ml ferulic acid for 24 h and subsequent exposure of the cells to 50 μM H_2O_2 for 30 min.

	DNA damage (% tail DNA)			DNA damage (% tail DNA)	
	Mean	SE		Mean	SE
Control	3.2	0.5	Control	2.6	0.3
H_2O_2	40.3	0.3	H_2O_2	42.1	2.7
P1	30.3	7.3	B1	25.8*	7.5
P2	29.0	6.0	B2	9.6*	1.1
P3	29.9	3.9	B3	16.0*	2.0
P4	23.1	3.2	B4	14.0*	3.8
FA	20.3*	5.7	FA	17.1*	0.8

Values are mean of three independent experiments. Statistical analysis was by ANOVA followed by Dunnett's test.
*Denotes significant difference ($P<0.05$) in DNA damage, relative to H_2O_2 control.

The total phenol content of P1, P2, P3 and P4 was 0.058, 0.533, 0.294 and 0.123 mg gallic acid equivalents (GAE)/ml, respectively. The total phenol content of B1, B2, B3 and B4 was 0.083, 0.732, 0.267 and 0.128 mg GAE/ml, respectively. The addition of 50 μM H_2O_2 for 30 min significantly increased ($P<0.05$) tail DNA to approximately 40% in U937 cells. The pale BSG extracts failed to significantly protect ($P<0.05$) against the oxidant induced DNA damage. However, all of the dark BSG extracts provided significant protection ($P<0.05$) against the damage induced by H_2O_2. In conclusion, it was shown that the dark BSG extracts show greatest antioxidant potential in U937 cells. Given that these extracts were found to have higher total phenol content than the corresponding pale BSG samples, a relationship between polyphenol concentration and protection against oxidant-induced DNA damage is evident.

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