Antioxidant Components of *Cichrium intybus in Vitro* and *in Vivo*

Hyun Jung Woo[a], Ji Hyang Kim[a], Deok Cho Yang[b], Jin Kyu Kim[a]
a Korea Atomic research Institute, Deajeon 305-353, Korea, jkkim@karei.re.kr
b Chungbuk National University, Cheongju 361-763, Korea

1. Introduction

Cichorium intybus is a perennial herb of the Compositae Family, native of Europe, west and central Asia, north of Africa and South America. The plant grows to a height of about 50-150 cm. The flowering time of it is from July to September. The flower is lingulate and blue. The basal leaves and the stems of the *C. intybus*, when they are very tender, are edible [1]. The root, dry and toasted, can be used as a substitute of coffee. Its tuberous roots store inulin as a reserve carbohydrate, which consists of a chain of fructose molecules with a termonal glucose molecule. It may be classified as a fructoligosaccharide, and can be found in more than 30,000 vegetal products. Amongst these vegetables, the chicory roots are outlined for the production of inulin on an industrial scale due to the root's stability in the production of long chains of GFn and constant growth, even in moderate climates. Its use in products with low calories and reduced fat levels, in Europe, the United States and Canada, is already quite widespread [2].

The present experiment was carried out to indicate that *C. intybus* contains both prooxidant and antioxidant compounds which can act *in vitro* and *in vivo*.

2. Methods and Results

2.1 Extracts of plants

The extract of *Cichorium Intybus* was kindly provided by Kyung-Won Synergy Co. Ltd..

2.2 Single Cell Gel Electrophoresis Assay

B16 melanoma cells were obtained from ATCC. B16 melanoma cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37℃ in 5% CO_2, 95% O_2 in a humidified cell incubator. We treated cells with the plants extracts. After treatments of the extracts, the cells were cultured in a CO_2 incubator for 1 hr prior to the addition of irradiation. The irradiated groups were exposed to γ-radiation from a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 76.8 Gy/hr [3]. Mean while, 100㎕ of the cell was then added to 1% of 100㎕ of a low melting point agarose. A second layer of 200㎕ of the sample mixture was poured out to the precoated slides. A third layer of 200㎕ of the LMA was poured out to the slides. The slides were placed in a prepared cold lysing solution for 1 h at 4℃ in darkness. After lysis, slides were then placed in an alkaline buffer for 5 min to allow for the unwinding of the DNA to occur. Electrophoresis was conducted for 20 min at 26 V and 300 mA. Slides were then drained, placed on a tray and washed slowly with three changes of 15 min each of a neutralization buffer. Slides were stained with ethidium bromide. Nuclei of 50 lymphocytes per slide per sample were examined at random using an epi-fluorescence microscope [4].

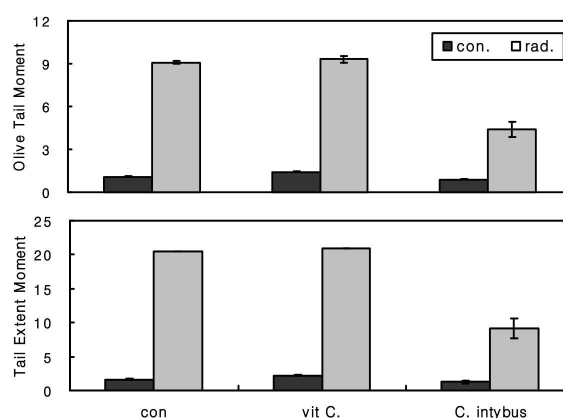

Figure 1. The in vitro genotoxicity assays of the irradiation and *C. intybus* treatment using comet assay of B16 cell. extent tail moment = tail length × tail % DNA /100, tail extent moment = tail length * tail % DNA/100.

In the *in vitro* comet assays for the genotoxicity in the B16 melanoma cells, the control group showed that more DNA breakage occurred in the irradiation group than in the extract-treated group. The tail extent moment and olive tail moment of the irradiation groups of control and ascorbic acid were 20.49±0.0063, 9.09±0.120, 20.87±0.000 and 9.31±0.233, respectively (Figure 1). In the case of the irradiation group pretreated with the *C. intybus* extracts, the tail extent moment and olive tail moment were 9.15±1.410 and 4.40±0.547, respectively. The group treated with the extracts of *C. intybus* exhibited lower damage than the control and vit C groups.

2.3 Animal treatments and Irradiation

The animal was purchased from the Daehan Biolink (Chungbuk, Korea) Fisher 344 rats (male, 5-week-old) were used throughout the investigations. The irradiated

groups were exposed to gamma-rays from a 60Co source with a total dose of 6.5 Gy. After treatment STZ and irradiation, the rats were allowed 0.5mg/ml and 1mg/mlextracts of C. intybus in water for 5 weeks. After 5 weeks, the animals were sacrificed by cervical dislocation. Levels of some enzymes were checked serum in experimental animals.

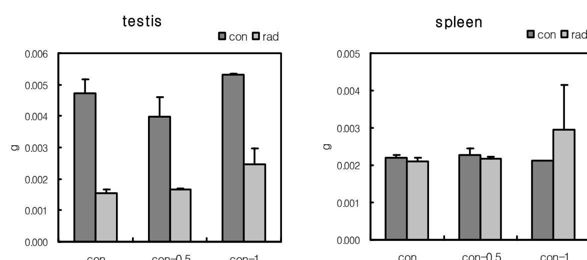

Figure 2. The effect of the extracts of C. intybus on the organ to body weight ratio.

The ratio of the weight of testis to the body weight in the irradiation treated groups was lower than that of the control group. But the ratio of the weight of testis to the body weight in the 1 % of *C. intybus* treated group was higher than those of the irradiation control group. The ratio of the weight of spleen to the body weight was not significantly different between the treatment groups and the control. That of 1% of *C. intybus* treated irradiation group was shown an error of the experiment (Figure 2).

Table 1. Relative value of the level of some enzymes in serum of the experimental groups.

		RBC (10^6/μL)	WBC (10^3/ μL)	ALP (U/L)
Con.	Con.	9.15 ± 0.16	4.15 ± 0.23	296.5 ± 3.54
	Rad	7.65 ± 0.22	1.83 ± 0.49	247.0 ± 41.01
0.5 % extract	Con.	8.46 ± 0.51	4.12 ± 0.08	310.5 ± 14.84
	Rad	6.88 ± 0.49	2.01 ± 0.40	256.5 ± 17.68
1 % extract	Con.	8.33 ± 0.70	3.99 ± 0.30	261.0 ± 15.26
	Rad	7.15 ± 0.74	2.25 ± 0.04	272.0 ± 11.31

RBC; Red blood cell, WBC; White blood cell, ALP; Alkaline phosphatase.

The blood assays were done to analyze RBC, WBC and ALP (Table 1). The RBC concentrations of all the irradiation groups showed lower value than that of the control group. Between the irradiation control group and the extract treated groups there was no significant difference. In the case of WBC, the *C. intybus* treated irradiation groups showed higher values than the irradiation control group. The ALP concentrations were not significantly different.

These experimental results have revealed that treatment of the extracts of *C. intybus* have the antioxidant effect in irradiation rats.

3. Conclusion

Cichorium intybus is a perennial herb of the Compositae Family. The present experiment was carried out to indicate that *C. intybus* contains both prooxidant and antioxidant compounds which can act in either chemical or biological systems. These results mean that the extracts of *C. intybus* have an excellent ability to scavenge radicals and thus can act as radioprotector.

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