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Studies on metabolic extract of *Alternaria alternata* and toxicity

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Abstract— *Alternaria alternata* were isolated from wheat seeds collected from villages in Tianshui area of Gansu Province where Kaschin-Beck disease is prevalent. TLC, IR and UV analyses of metabolic extract of *A. alternata* show that *A. alternata* produce main secondary metabolites, including alternariol, alternariol methyl ether, and tenuazonic acid.

There was a significant decrease in Se-GSH-Px and SOD activities, and an acceleration in lipid peroxidation in rats fed with a diet containing metabolites produced by *A. alternata*.

Keywords: Kaschin-Beck disease; *Alternaria* toxins; lipid peroxidation; Se-containing glutathione peroxidase; superoxide dismutase.

INTRODUCTION

The *Alternaria* produce numerous secondary metabolites, including tenuazonic acid, alternariol, alternariol methyl ether, altenuene, and altertoxins I, II and III. The Ames *Salmonella typhimurium* assay was used to demonstrate that an extract of the mold *Alternaria alternata* was mutagenic. The mutagenic extract was fractionated, and altertoxins I, II and III were isolated. Altertoxins I, II and III were mutagenic to *S. typhimuria* TA98, TA 100, and TA1537 with and without metabolic activation (Stack, 1986; John, 1984). Tenuazonic acid produced by *A. alternata* can inhibit protein synthesis in mammalian tissue (Meronuck, 1972; Carraxso, 1973). Riess showed that Alternariol and Alternariol monomethyl ether could be produced from wheat by *A. alternata* on it (Reiss, 1983). Alternariol monomethyl ether is particularly important because a recent study indicated it has weak mutagenic activity (Scott, 1980). Bai Fengyan *et al.* (Bai, 1990) reported that *A. alternata* was the predominant fungus isolated from the wheat seeds, the samples were collected from 10 villages in Tianshui area of Gansu Province where Kaschin-Beck disease is prevalent and wheat is the major food for the inhabitants in Tianshui endemic area.

In view of the evaluation of the potential hazard of *Alternaria* in feeds and feedstuffs, and of the possibility that *Alternaria* metabolites might be responsible for the occurence of Kaschin-Beck disease in Tianshui area, a project was undertaken to obtain *Alternaria* isolates from wheat and

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corn inoculated, in order to determine which metabolites they produce, and to test the extracts for toxicological effects by using Wistar rats to examine the effects of metabolites produced by *A. alternata* on lipid peroxidation, Se-containing glutathione peroxidase (Se-GSH-Px) activity and superoxide dismutase (SOD) activity.

MATERIALS AND METHODS

Isolation of A. alternata and cultural conditions

Alternaria alternata organisms were isolated from wheat seeds collected from villages in Tianshui area of Gansu Province where Kaschin-Beck disease is prevalent. To test for the production of metabolites, *A. alternata* was cultured on wheat or corn. Flasks containing 100 g of wheat or corn and 60 ml of water were autoclaved at 121°C for 20 min at 1.1 kg/cm^2. The sterilized substrate was inoculated with spores of *A. alternata* and incubated without shaking for 18 days at 28°C in the darkness.

Extraction

100 g cultures were acidified with 100 ml pH 2.0 chlorohydric acid solution and ground before it was extracted twice each with 250 ml chloroform. The chloroform extracts were combined and concentrated on a rotary evaporator to yield extract as a homogeneous oil.

TLC analysis of extract

Extract was analyzed qualitatively by thin layer chromatography (TLC). Extract was dissolved in chloroform, then a 10 μl portion was spotted on a silica gel TLC plate (GF). After the plate was developed with benzenemethanol acetic acid (90: 5: 5) and dried, then detected under longwave UV light (3650 Å) and shortwave UV light (2537 Å).

Ultraviolet spectral (UV) and infrared spectral (IR) analysis

Spectral absorption data were obtained on a Shimadzu Model UV-3000 UV/ vis spectrophotometer with an extract solution of chloroform-ethanol (1: 1, V/ V) for the UV absorption, and a Digilab Fourier Transform Infrared spectrometer FTS-20/ 90 with KBr disk for the IR. KBr pellets were prepared in a usual way.

Animal experiments

In view of mammalian toxicity and natural occurrence of *Alternaria* toxins in feed and food, Wistar rats were used to examine the effects of metabolites produced by *A. alternata* on lipid peroxidation, Se-GSH-Px and SOD activities.

Experiments were performed with male rats each having a weight of 180－200 grams, by supplying rats with alternaris toxins in drinking water for 14 days. The treated groups were administered with 0.1, 0.2 and 0.4 ml extracts, respectively, in addition to basal diet and normal drinking water. The control group was supplied with basal diet and normal drinking water.

Se-GSH-Px activity was assayed by the method of Hafeman (Hafeman, 1974).

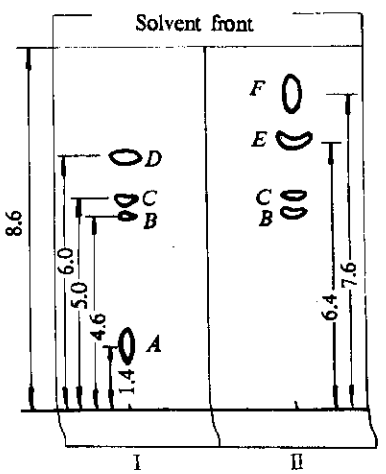
The fluorescent product of lipid peroxidation was assayed by RF-520 fluorometer according to the method of Fletcher (Fletcher, 1973).

SOD activity was assayed by the method of Tang Jiajun (Tang, 1985).

RESULTS AND ANALYSES

TLC, IR and UV analyses of extracts

Extracts were analyzed qualitatively by TLC. Thin-layer chromatomap (Fig. 1) shows that spot D (*Rf* 0.69) and spot A (*Rf* 0.16) were detected as blue fluorescent spots under longwave UV light, spot F (*Rf* 0.88) was detected as a darkspot under shortwave UV light, it has been reported that alternariol (*Rf* 0.16) and alternariol monomethyl ether (*Rf* 0.69) were detected as blue fluorescent spots under longwave UV light, tenuazonic acid (*Rf* 0.88) was detected as a dark spot under shortwave UV light (Stack, 1986).



	A	B	C	D	E	F
Migratory distance, cm	1.4	4.6	5.0	6.0	6.4	7.6
Rf	0.16	0.53	0.58	0.69	0.74	0.88

Fig. 1 The TLC of alternaria extract

I: A, B, C, D were detected as blue fluorescent spots under longwave UV light (3650 Å)

II: B, C, E, F were detected as dark spots under shortwave UV light (2537 Å)

The metabolites of *A. alternata* cultured on wheat or corn in the same way as described above have no obvious difference from each other in their IR spectra (Fig. 2). The IR spectra of alternariol and alternariol methyl ethers show carbonyl bands ranging from 1655 cm^{-1} for hydrogen-bonded σ-lactones or esters to 1720 cm^{-1} for the fully methylated products.

Altertoxins I, II and III have hydroxyperylenequinone structures which occur at 1800－1600 cm^{-1} (Coombe, 1970; Stack, 1986). Extracts absorb strongly in the 3400 cm^{-1} region, which exhibits the presence of hydroxyl groups. The methylene absorption at around 2900 cm^{-1} strong. Carbonyl absorption occurs at 1722 cm^{-1}. Benzene ring skeleton structure occurs at 1450 cm^{-1}.

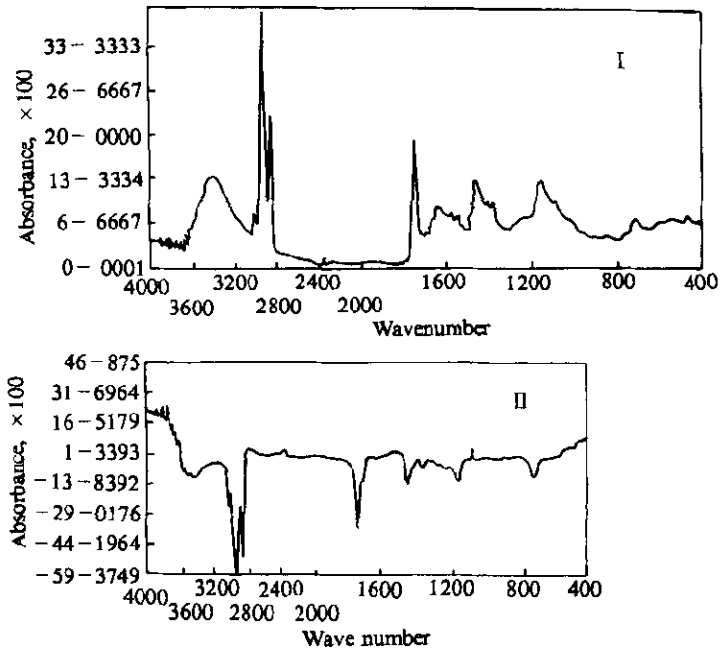


Fig. 2 The IR spectra of *Alternaria* extract
I: *A. alternata* was cultured on wheat
II: *A. alternata* was cultured on corn

Extracts show UV spectra with maxima in the regions of 355, 300, 290, and 260nm (Fig. 3), these are similar with respect to the related compounds alternariol and alternariol mono- and trimethyl ethers (Coombe, 1970).

TLC, IR and UV analyses of metabolic extract of *A. alternata* show that *A. alternata* produce main secondary metabolites, including alternariol, alternariol methyl ether, and tenuazonic acid.

Effects of metabolites produced by A. alternata on lipid peroxidation, Se-GSH-Px and SOD activities

Free radical formation and lipid peroxidation have been postulated as important factors causing membrane damage and accelerating the aging process. One of important Se-GSH-Px functions is the prevention of oxidative damage to cellular membranes and other sensitive cellular

sites. The variation in human erythrocyte GSH-Px activity as a result of cellular aging was examined by Perona *et al.* (Perona, 1978).

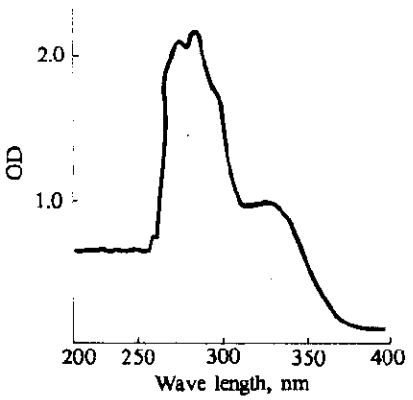


Fig. 3 The UV spectra of *Alternaria* extract

Results (Table 1) show that there was a 14% increase in fluorescent lipid peroxidation products from serum of the treated group administered with 0.4 ml *Alternaria* extract. There was a significant decrease in the blood Se-GSH-Px activity of extract-administered rats as compared to the control group, blood Se-GSH-Px activities decreased by 9.4%, 15.7%. and 45.7% in the rats administered with 0.1. 0.2 and 0.4 ml *Alternaria* extract, respectively.

Table 1 Effects of metabolites produced by *A. alternata* on lipid peroxidation and Se-GSH-Px activity

Group*	Flourescent products from serum, relative, %	Blood Se-GSH-Px activity, relative, %
1	100	100
2	100.8	90.6
3	98.7	84.3
4	114.0	54.3
5	88	99.7

* Group is the same as that in Fig. 4

It has been reported that a significant positive correlation existed between $O_2^{\cdot-}$ and SOD activity. Fig. 4 shows that SOD activity was significantly lower in liver of rats administered with 0.4 ml *Alternaria* extract as compared to the control, particularly to the group by supplementing selenocarrageenan selenium.

Results from our study indicate that there were a significant decrease in Se-GSH-Px and SOD activities, and an acceleration in lipid peroxidation in rats fed with a diet containing

metabolites produced by *A. alternata*. This result is consistent with the observation on oxygen radicals damage in patients with Kaschin-Beck disease. The data presented provide evidence that supplements of selenium has been proved to be highly effective in preventing the toxicity of *Alternaria* extract.

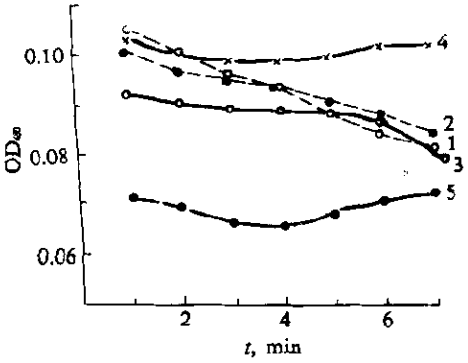


Fig. 4 The effect of metabolites by *A. alternata* on SOD activity
Group 1. contrast 2. 0.1 ml *alternaria* toxins administered
3. 0.2 ml *alternaria* toxins administered 4. 0.4 ml *alternaria* toxins administered
5. 0.4 ml *alternaria* toxins and 0.25 ppm Se of k-selenocarrageenan administered

Further work is necessary for a better understanding of the correlation between Kaschin-Beck disease and metabolites produced by *A. alternata*.

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