The Inhibitory Effect of Natural Plant Extract AMH on the Mutagenicity of Several Carcinogens in Vitro

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Abstract **Objective** To observe the antimutagenic effect of natural plant extract(AMH) on 2-aminofluorene (2-AF), benzo(a)pyrene (B(a)P), aflatoxin B1 (AFB1), cyclophosphamide (CP), cigarette smoking condensate (CSC), hair coloring cream (HCC), dimethylnitrosamine (DMN) and diethylnitrosamine (DEN). **Methods** Salmonella typhimurium/mammals microsomal enzyme test (Ames test) was used. **Results** The AMH could markedly inhibit the mutagenicity of those carcinogens and mutagens common in human living environment. The inhibitory rates were 80%~100%, and good dose-effect relationships were presented. **Conclusion** The study proves that AMH is able to significantly inhibit the mutagenicities of the tested 8 carcinogens and mutagens.

Key Words Antimutagenicity; Tumor Prevention; Biologic Resource; Mutagenicity

With the increasing of industrial pollution and environment pollution, people expose to more and more carcinogens in their occupational and living environment. The incidence of tumors in human is getting higher. Tumor is one of the main causes in human death. The main mechanism of tumor occurrence is that the chemical carcinogens have mutagenic effect to genes [1]. Tumor prevention is an important measure to decrease the incidence of tumor. Tumor-chemoprevention is an important measure in tumor-prevention[2]. Yunnan province is located in the southwest of China. Because of its special geological and climate condition, it possesses a lot of unique biological resources. We found that the extract of one kind of unique fungi plant could strongly inhibit the mutagenicity of several carcinogens and mutagens common in living condition. The following is the result of the study.

MATERIAL AND METHODS

The natural plant extract AMH (Antimutagenic Herb) It is extracted from one kind of unique fungi plant (one kind of wild mushroom, the exact formal name is unknown). The extract is a water-soluble liquid. It is red and clean, without odd smell and taste. 1ml extract equals to 0.25g dry fungi plant.

Carcinogens and mutagens Benzo(a)pyrene, 2-aminofluorene, Dimethylnitrosamine and diethylnitrosamine were ordered from Sigma co., USA. Aflatoxin B1 was provided by China Preventive Medicine Academy. Cyclophosphamide was produced by Jiangshu Hengri Medicine Company, China. Cigarette smoking condensate was provided by Yunnan Tobacco Academy. Hair coloring cream was made in Japan. The tested dose of B(a)p and AFB1 was both 1μg/plate, CP was 200μg/plate, CSC was 500μg/plate, HCC was 2000μg/plate, and both DMN and DEN were 400μg/plate.

Method According to the antimutagenicity test methods made by the Health Department and the GB15193.4-94 of China [3], referring to other antimutagenic reports[4,5], we used the Salmonella typhimurium/mammals microsomal enzyme test (Ames test) for the antimutagenicity evaluation. The main procedures are as following: 0.1ml AMH (or 0.1ml distilled water in the positive control group), 0.1ml positive mutagen, 0.1ml cultured bacterial and 0.5ml S9 mix were correspondingly added into the 2.5ml top agar culture. All were fully mixed and were poured on the agar culture. The plates were cultured at 37℃ for 48 hours. The revertant colonies were counted. 3 parallel plates and repeated test were done. DEN and DMN were pre-activated before used.

Tested bacterial Strains TA97, TA98 and TA100 were used in the test. All these strains´ characters were identified before used. Because all of the positive mutagens needed activated, the test was done under S9 system.

Statistics SPSS statistics soft-ware and Q test were used.

RESULTS

AMH could inhibit the mutagenicity of all the 8 tested positive mutagens in Ames test. The results were shown in table 1 and table 2.

According to the results shown in table 1 and 2, AMH could strongly inhibit the mutagenicity of the 8 carcinogens or mutagens. The highest inhibitory rate was 100%, most were between 80% ~100%. The revertant colonies of the AMH group was significantly lower than those of the positive control group. The results showed AMH was an effective natural antimutagen.

In order to further study the antimutagenicity of AMH, especially the dose–effect relationship, we studied the antimutagenic effect of different doses of AMH on the mutagenicity of 2–aminofluorene. The result was shown in table 3.

The result of table 3 showed that AMH could markedly inhibit the mutagenicity of 2–AF, and good dose–effect relationship existed in its antimutagenicity. The result further indicated that AMH was a strong natural antimutagen.

Table 1. The antimutagenicity of AMH (the first time, average colonies of 3 plates)

mutagen	dose μg/plate	TA97			TA98			TA100		
		–AMH	+AMH	IR(%)	–AMH	+AMH	IR%)	–AMH	+AMH	IR%)
2–AF	10	2687.3	655.7	80.6	>4000	864.3	79.0	1093.0	225.7	87.9
B(a)P	1	641.7	290.3	73.7	306.7	59.0	90.5	388.7	147.3	85.3
AFB1	1	————	————	————	283.3	63.3	87.9	————	————	————
CP	200	————	————	————	————	————	————	491.7	179.3	81.0
CSC	500	441.0	218.0	80.9	189.0	31.7	100.0	106.0	104.7	6.5
HCC	2000	>5000	662.7	89.7	>5000	811.7	84.3	672.0	138.3	94.3
DMN	400	————	————	————	94.0	48.7	74.3	————	————	————
DEN	400	740.3	496.0	42.5	————	————	————	————	————	————
Spontaneous	————		165.2			33.0			105.8	

IR: inhibitory rate, IR= (G–H)/ (G–I) ×100%; G: the colonies of the control group (–AMH), H: the colonies of the AMH group (+AMH); I: the colonies of the spontaneous group.

Table 2. The antimutagenicity of AMH (the second time, average colonies of 3 plates)

mutagen	dose μg/plate	TA97			TA98			TA100		
		–AMH	+AMH	IR(%)	–AMH	+AMH	IR(%)	–AMH	+AMH	IR(%)
2–AF	10	1268.0	145.0	899.8	2228.7	169.0	93.7	893.3	115.3	98.4
B(a)P	1	437.7	165.3	92.3	289.7	51.0	92.4	265.3	132.7	81.5
CP	200	————	————	————	————	————	————	526.7	167.3	84.8
CSC	500	456.3	198.3	82.3	203.3	36.7	96.9	————	————	————
HCC	2000	>5000	323.7	96.3	>5000	215.7	96.3	568.3	129.7	94.2
DMN	400	————	————	————	103.7	56.7	64.9	————	————	————
DEN	400	687.7	435.3	46.3	————	————	————	————	————	————
Spontaneous	————		142.7			31.3			102.7	

Table 3. The antimutagenic effect of AMH on 2–AF ($\bar{X}$±S)

AMH (concentration)	2–AF μg/plate	TA98		TA100	
		colonies	IR(%)	colonies	IR(%)
0	10	1568.7±123.7	----	795.3±56.2	----
1	10	154.3±13.2	92.2	125.7±6.5	98.7
1/2	10	190.7±4.7	89.8	204.7±12.2	87.0
1/4	10	281.0±11.5	83.9	278.3±10.3	76.2
1/8	10	483.3±7.8	70.8	367.7±21.8	63.0
Spontaneous	---	34.7±1.2	----	116.7±5.6	----

DISCUSSION

Cancer is the main disease which causes human death. It is mainly caused by chemical carcinogens existing in our environment. It is known that tumor chemoprevention may play an important role in tumor prevention [2]. The study of cancer–prevention medicine is a prosperous area in cancer research. many studies have been done in this region [6,7,8] . Reports showed that some biological resources may have antimutagenicity, or tumor–prevention, or anti–cancer components. These components have good antimutagenicity or tumor prevention effect.

Yunnan province possesses a lot of unique biological resources, especially a lot of fungi plants. Some of them have special medical functions. AMH was extracted from one kind of wild mushroom. AMH has strong antimutagenic effect on the mutagenicity of carcinogens and mutagens. But the functional components and its antimutagenicity mechanism are unknown yet. These will be further studied in our later research. AMH has been proved without any toxicity in our other tests. In another study, we also found that AMH could strongly inhibit the incidence of tumors induced by benzo(a) pyrene in mice. AMH may have great potential in human cancer prevention.

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