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THE ACUTE TOXICITY AND BONE-MERROW MICRONUCLEUS TESTS OF WATER EXTRACT FROM *Avicennia marina* FRUITS IN MICE

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ABSTRACT

The acute toxicity and mutagenesis of water extract from Avicennia marina fruits in mice were studied with the oral acute toxicity test according to Karber's method and the bone-marrow micronucleus test for mutagenic observation.The results showed the water extract from A. marina fruits presented theacute per oral toxicity at LD_{50} >10.000 mg/kg; the bone-marrow nicrobucleus test indicated no mutagenic effects. Water extract from A.Marina fruits belong to non toxic compounds and has no mutagenic effects under the experimental conditions.

Key word*: Water extract from Avicennia marina fruits; Acute toxicity test; bone-marrow micronucleus test; mutagenesis*

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INTRODUCTION

Avicennia marina (Forsk) Vierh is one of the widest distributed mangrove tree species in the coastal mudflats of world tropics and subtropics and can be found as a poineer tree species in coastal wetlands of Hainan, Guangdong, Guangxi and Fujian in China. The fruit of the species 1 – 2 cm in diameter and 2-3g/fresh, fruiting in the months between June and September People in the coastal areas in S. China with the species distributed collected the fruits for food or fodder, and their experience showed that the fruits collected in mudlfats are soft and without much bitter taste while in sandbeach harder and litter bit more bitter. The fresh fruits are more bitter while before boiled and soaked in water for 1-2 days as edible food while most of the contained tanin eluviated during the process. We examined nutrient contents of the fresh fruits and treated friuts collected from Techeng Isle, Zhanjiang, China, showed that it were high in nourishment value as it had less tanin content compared with fruits of other mangrove species. Collected as folk food material, the *A. marina* fruit had high contents of raw starch, total soluble sugar and raw fiber but low content of raw protein, raw fat and pectin, and had certain tannin **(Table 1).**

MATERIALS AND METHODS

The preparation of water extract from A.marina fruits

The fruits of A.Marina were collected in the

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Techeng Isle, Zhanjiang, China, with nutrient content showed in table 1. The fresh fruits steam boiled at 95^{o}C for 8-10 minutes and soaked intap water at room temperature for 36h,then air dried and smashed to pass the 630μm screen in order to obtain the fruit powder. 50g fruit powder were soaked in 500ml distilled water for 1.5h and boiled for 1h,then filtrated for collection of extract, the left add 400ml more ditilled water and boiled for 1h and then filtrated for another collection of extract, combined the extracts for concentrating to 50ml as 1g/ml fruit powder content in the final water extract from the *A.marina* fruits.

Table 1. Nutrient contents in fruits of *A. marina* collected in Techeng Isle, Zhanjiang, China(%)

Tested Item	fresh fruits			treated fruits*		
	full fruit	flesh	pericarp	full fruit	flesh	pericarp
water	63.25	60.50	76.00	10.84	8.51	8.12
ash	1.81	2.13	1.21	4.18	3.95	3.83
raw protins	0.92	0.61	0.48	0.68	0.54	0.49
raw fat	0.48	0.37	0.35	0.16	0.07	1.2
raw starch	15.38	9.75	10.69	37.17	34.42	40.07
soluble sugar	9.44	18.04	9.39	47.90	49.98	14.00
raw fiber	3.07	1.03	1.87	4.23	3.09	13.55
tanin	0.63	1.85	1.15	5.27	5.41	1.67
pectin	0.32	0.34	0.13	3.08	1.19	3.31

*The fresh fruits steam boiled at 95℃ for 8-10 minutes and soaked in tap water at room temperature for 36h then air dried.

The test animal

200 individual KM white mice, normal grade, with body weight 18 – 22 g and 25 – 30 g, half male and half female, bought from 200 individual KM The Test Animal Center og Guangxi Midical University, China (ID : Gui-SCXK2003-0003), I week feeding before test under test under controlled air temperature at 25^{o}C – 28^{o}C and air humidity at 60% - 80% without convection wind and the full nutrient fodder provider by Guangdong Medical College were used with distilled water freely eaten by the mice and the fodder containers were changed each day.

Test equipments

One small smashing machine,one PHS-3C digital pH detecter,one YXQ-SG46-280S High-pressure steam sterilizer,some medical scissors and pincers, one set microscopes with oil immersion lens,one cell number reader, enough white mice feeders provided by Guangdong Medical College,China.

The chemical agents

Ethanol (pure), methanol (pure), glycerol (pure), young cow blood serum (provide by Changhum Bao Tai Biotech Limited Co, cylophosphamide (provide by Jiangsu Henrui Medical Limited, Co., 10% PBS Giemsa staining liquid (Giemsa stain 1g + glycerol 66 mL + methanol 60 mL (pH 6.4).

Test Method

Acute peroral toxicity test [1,2]

The small white mice weight 18-22g were divides into random 5 groups, each with 5

male and 5 female individuals respectively, the neigbouring groups feeding difference at 1:0.7, as 20.000 mg/kg, 14.000 mg /kg, 9.800 mg/kg, 6.860 mg/kg, 4.802 mg/kg as the final fruit water extract volume to mice body weight respectively. The peroral toxicity test feeding volume was 0.2ml/10g body weight, and no food feeding 8h before the test feeding, then continuing 1 week observation during the test, recorded the mice poisoning symptoms and dead number(Table 2), then calculated LD50.

Table 2. The tested mice acute toxicity observation indicators

Item	behave and symptom
Spontaneous acts	increase, decrease, flee,sleeping
Muscle movement	thrill, twitch, palsy, ataxia
Muscle strain	improved, weakened, strong and straight, flab
Reaction	jitter, lagging
vegetative nerve	erect hair, weep, salivating, protruding eyes, diarrhoea, wrest body
Breath	retrained, fast, weakened
Skin color	pale, violet, hyperaemia
Dieing time	acute, slowly
death symptom	struggle, chiao, foaming

Bone-marrow micronucleus test [3]

Divide 50 samll white mice weight 25-30g into 5 groups evenly, half female (f) and half Male (M). Group I as negative check. Group II, Group III, Group IV were feeded with different amount og the fruit water extract and Group V as positive check. The negative check were feeded with salt water (SW) and positive check were feeded with cyclophosphamide (CP) on the test via Oral injection at 5.000 mg/kg, 2.500 mg/kg and 1.250 mg/kg as the final fruit water extract volume to mice oral injection with interval 24h. Doffing the necks of mice after 6h later from the second injection, then cut 2 pieces of side femur to make bone marrow smear at glass flakes. Use methanol for 10minutes and then 10% PBS Giemsa staining liquid(pH6.4) to fix and stain the dried bone marrow smears for double-blind study: Read 1000 PECs number and the number(num.) of PECs with micronucleus among them for each smaer so as to obtain the rates of PEC micronucleus(PE Cm ‰). The t test was applied for the inter group comparison[4].

RESULTS AND DISCUSSION

Results

Aacute peroral toxicity test

The test result showed that the tested mices showed no abnormal symptoms in spontaneous acts, muscle movement, muscle strain, reaction, vegetative nerve, breath, skin color, and they were back to quiet 1h after feeded and drank water normally, without sign of dieing during the test period before the 8th day killed to examine internal symptoms. The killed body anatomy showed normal stomach without flatulent phenomenon, and all other organs, heart, liver, spleen, lung, kidney, adrenal gland, thymus gland, ovary, uterus, testicle, intestines and thorax, abdominal, observed by naked eye, were no abnormal, with highest dose feeding group Weighted 25.53± 1.31 g/individual after the test 22.72±1.37 g / individual. The acute per oral toxicity LD_{50} > 10.000 mg/kg showed that water extracts from fruits of *A. marine* were actually non toxic grade.

Table 2. The statistic data of the micronucleus test of water extract from *A .marina* fruits in mice

Group	Extract Dosage (mg/kg)	Individual Num.		PCE Num.		PECm Num.		PECm rate (‰)	
		F	M	F	M	F	M	F	M
I	SW	5	5	5000	5000	27	27	5.4	5.4
II	5000	5	5	5000	5000	35	37	7	7.4
III	2500	5	5	5000	5000	37	29	3.7	5.8
IV	1250	5	5	5000	5000	24	30	4.8	6
V	100 (CP)	5	5	5000	5000	115	119	23**	23.8**

Note : All recorded rates were compared with the rates of the negative group 1, ** < 0.001 as showed in the Table 2, all other recorded rates compared with negative group 1 were no significant differences (P>0.005), but there were significant differences between the groups of the negative and positive (p<0.01), and no significant differences between different dosages used, showing the test mice were high sensitive and the test results were reliable: The result of mice bone –marrow PEC micronuleus test was negative test

Discussion

Mice bone marrow polychromatic erythrocyte(PEC) micronucleus test mainly evaluates the change of the chromosome structure, while micronuclues are small chromosome particles remianed in cytoplasm of daughter cell while the most of chromosome materials entering new daughter cell's nuclues at late stage of mitosis. The micronucleus is same in structure as the chromosome in nucleus of the same daughter cell. The appearance of it is corelated with chromosome aberration. So the examination of cell micronucleus could uncover the chromosome integrality affected by tested chemical or other materials/factors as whether the influenced chromosome Could separate normally or be introduced some mutagenesis [5-6]

CONCLUSION

This study showed that the fruit water extract from *A.marina* were actually no toxic grade and no chromosome mutagenesis assessment based on the results of the mice acute peroral toxicity test and the mice bone marrow PEC micronucleus

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