

Antistress effect of oligosaccharides extracted from *Morinda officinalis* in mice and rats

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KEY WORDS *Morinda officinalis* How; oligosaccharides; stress; testosterone; corticosterone; immunity

ABSTRACT

AIM: To explore the antistress effect of *Morinda officinalis* (Chinese medicine "Bajitian") oligosaccharides (MW-97) in mice. **METHODS:** Male mice and rats were subjected to a variety of unpredictable stressors on a daily basis over 15 d period and then the Vidiomex-V image pattern analytic system was used to observe the spontaneous motor activity. Meanwhile, regular method was used to prepare pathologic section of adrenal gland and blood cells analytic system was used to detect white blood cell (WBC) count (including relative WBC percentages) in peripheral blood. On the end, the serum level of stress hormone was detected using radioimmunoassay. **RESULTS:** Chronic stress resulted in diffuse hyperplasia of the adrenal cortex and atrophy of the adrenal medulla in mice, which suggested that stress-adaptation failure of the adrenal gland occurred, while adrenal gland of the mice pretreated with MW-97 (100 mg/kg, ip) prior to each stressor for 15 d did not occur any pathologic changes. In addition, chronic stress also significantly reduced the WBC count and relative WBC percentages in the peripheral blood, including the percentage of lymphocytes, monocytes, neutrophils, however, MW-97 (25 and 100 mg/kg) reversed these changes and raised WBC count, along with relative WBC percentages significantly. Furthermore, the serum concentration of testosterone was decreased and corticosterone was increased significantly in chronically stressed animals. MW-97 also declined the serum level of corticosterone and raised level of testosterone. MW-97 had no effects on the spontaneous motor activity in the stressed mice. **CONCLUSION:** MW-97 had antistress

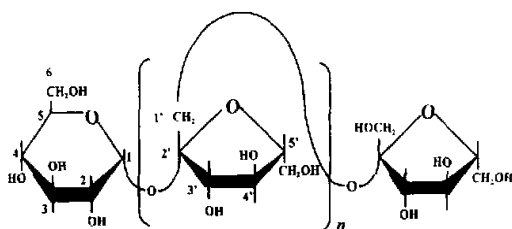
effect against chronic stress. moreover, MW-97 had no excitatory or inhibitory effects on the CNS, which suggested that MW-97 might become a new kind of antistress agent.

INTRODUCTION

Although clinical depression can be subdivided into several categories based on their etiologies, there are reasons to believe that the endogenous depression may be precipitated by environmental factors such as stress. It is well known that the inability to successfully cope with or adapt to stress can lead to ulcers, heart diseases, hormonal imbalances, pathologic changes, and affective disorder, etc. Current antistress drugs (eg, desipramine) had notable toxic or side effects. So, to develop powerful and low toxic antistress agents became an important researching direction in psychopharmacology.

Chinese traditional tonic medicine Bajitian (*Morinda officinalis* How), which is in common use has many effects, including tonifying the kidney, strengthening yang-qi, relieving rheumatism, etc.^[1]. In our previous study, it was found that the inulin-type oligosaccharides (MW-97) extracted from Bajitian had antidepressant effects, while little was known about its antistress properties. MW-97 was the mixture of several inulin-type oligosaccharides with similar structure shown below, it was the effective component. It is known that the key character of depression or chronic stress was hyperaction of the hypothalamo-pituitary-adrenal (HPA) axis and serum main glucocorticoids-corticosterone (Cort) is persistently elevated, then the neuroendocrine immunomodulating network would be in disorder, which was expressed with defection in sexual function and immunity as well as learning and memory, depression and dementia, etc. As the key effector of the HPA axis, the adrenal gland would also appear compensative pathologic changes. In this study, the chronic stress models in murine were established and the antistress effect of MW-97 was observed.

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Structure of MW-97 ($n = 2 - 5$)

MATERIALS AND METHODS

Animals Male mice (20 g \pm 2 g) of Kunming strain or male Wistar rats (180 g \pm 20 g) were provided by the Animal Center of Academy of Military Medical Sciences (Grade II, Certificate No 99001).

Drugs and reagents Bajitian oligosaccharides (MW-97, yellow powder, purity of oligosaccharides beyond 90%) was provided by the Botanical Chemistry Lab in our institute, desipramine (DIM) were from Sigma (USA); testosterone or corticosterone radioimmunoassay kit were from Academy of Chinese Atomic Energy Sciences.

Drug treatment Forty animals were randomly divided into five groups (eight mice in each group) according to their body weights as normal control, chronic stress control, drug treatment groups (DIM 15 mg/kg, MW-97 25 and 100 mg/kg, ip) respectively. Drugs were injected daily 30 min before each stressor and the control groups received only water injection daily.

Chronic stress procedure in mice^[2,3] The chronic stress regimen used was a variant of Roth *et al.*^[4]. Stressors were administered once per day over a period of 15 d between 8:00 am to 11:00 am. The exact order of stressors was detailed in Tab 1. The spontaneous motor activity of the mice was detected between 9:00 am to 11:00 am and the tail vein blood sample 20 μ L was collected between 2:00 pm to 4:00 pm on d 16, using the F-800 blood cell analysis apparatus (Sysmes Co, Japan), white blood cells (WBC) count was proceeded. On d 17, after sacrifice a trunk blood sample was collected and allowed to clot at room temperature, at the same time, adrenal gland was removed. The blood was centrifuged at 130 \times g for 10 min, and the supernatant was removed and stored at -20 $^{\circ}$ C until radioimmunoassay of testosterone was performed. The adrenal gland was put in 10% formalin solution for fixation, and pathologic section was prepared with regular method including dehydration, transparency, embed-

ment, slicing, and HE dyeing, finally, pathologic change was observed and taken pictures with microscope.

Tab 1. Chronic stress regime of mice.

Day	Treatment	Duration
1	Cold swim (12 $^{\circ}$ C)	6 min
2	Food deprivation	24 h
3	Foot shock (1 mA, 1 s duration, average 1 shock/min)	30 min
4	Overhang (2 - 3 cm apart from surface of water)	30 min
5	Water deprivation	24 h
6	Tail pinch (1 cm apart from the end of the tail)	1 min
7	Overhang	1 h
8	Foot shock	45 min
9	Cold swim	6 min
10	Water deprivation	24 h
11	Overhang	1.5 h
12	Food deprivation	24 h
13	Tail pinch	2 min
14	Foot shock	1 h
15	Cold swim	6 min

Chronic stress procedure in rats^[2-4] The regime of stress in rats was similar to that described in mice, the following two stressors were added; 2 h restraint and 60 min high speed horizontal shaking, while the stressor of overhanging in mice was not used in rats. Rats were killed by cervical dislocation 24 h after the last treatment and the serum samples was prepared and stored at -20 $^{\circ}$ C for radioimmunoassay of corticosterone.

Spontaneous motor activity Thirty minutes after drug injections, stressed mice were plunged individually into an open field (35 cm \times 30 cm \times 22 cm) fitted with a black rubber floor. Four mice was always performed in one period. The videotape scene was shown by computer display unit and Vidiomex-V image pattern analytic instrument (Columbus Co, USA) was operated and controlled by the computer. After subjects were placed in the open field, allowed to habituate to the environment for 10 min, subsequently the Vidiomex-V system would automatically display and record the spontaneous movement in later 10 min. The detected parameters included travelling distance, ambulatory time, resting time, and average speed.

Radioimmunoassay of testosterone and corticosterone The operating procedure was performed according to the specification of radioimmunoassay kit.

Statistics Values were given as $\bar{x} \pm s$. Com-

parisons of groups were made with one-way ANOVA analysis.

RESULTS

Pathologic change of adrenal gland in stressed mice The pathologic section of adrenal glands in normal control mice showed distinct configuration in an order arrangement of which was globular band, cortex fascicular band, and medulla respectively from outside to inside (Fig 1A). In chronically stressed mice, it was shown that cortex of the adrenal gland was diffuse hyperplastic and the medulla was atrophic (Fig 1B), which may be caused by excessive secretion of corticosterone in cortex and depletion of adrenalin vesicles in medulla. While adrenal gland of stressed mice treated with DIM 15 mg/kg was improved obviously, even to be normal (Fig 1C). The adrenal gland of mice treated with MW-97 100 mg/kg did not display any pathologic changes (Fig 1D). It was indicated that DIM and MW-97 had antistress effects.

WBC count in stressed mice One of the characters of stress or depression was immunity deflection which was mediated by high blood level of corticosterone. Our results showed that the counts of total WBC, lymphocytes, monocytes, and neutrophils were all decreased significantly. MW-97 at the doses of 25 and 100 mg/kg could raised them (Tab 2), which suggested that MW-97 might improve immunosuppressive states induced by corticosterone. These results supported antistress effect of MW-97.

Modulation of MW-97 on serum testosterone and corticosterone in stressed mice and rats

Compared with normal mice group, the weight of testicle of chronically stressed mice showed decreasing tendency, but there was no significant difference. While serum level of testosterone in stressed mice was reduced, the

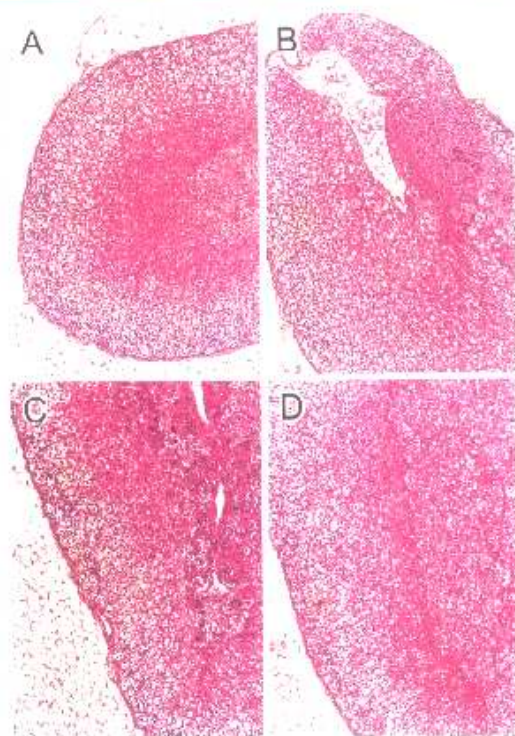


Fig 1. Effects of MW-97 and DIM on the pathologic change of adrenal gland in stressed mice. A: normal control; B: stressed control; C: stress + DIM (15 mg/kg); D: stress + MW-97 (100 mg/kg). HE stain, $\times 90$.

treatment of MW 97 at the doses of 25 and 100 mg/kg made it elevated (Tab 3) and decreased the serum level of corticosterone in chronically stressed rats (Tab 4), which suggested that MW-97 had antistress and Yang-tonic effects.

Spontaneous motor activity in stressed mice

The spontaneous movement of mice included walking, scratching, washing face, licking, and smelling, etc. In this test, all kinds of the parameters of spontaneous motor activity between stressed and normal mice had no

Tab 2. Effect of MW-97 on WBC count in stressed mice. $n = 8$. $\bar{x} \pm s$. $^b P < 0.05$ vs stress control. $^c P < 0.05$, $^d P < 0.01$ vs normal control.

Groups	Dose /mg·kg ⁻¹	Total WBC	Lymphocytes $\times 10^{-9}/L^{-1}$	Monocytes	Neutrophils
Normal	-	7.8±0.8	6.0±0.5	0.30±0.11	1.5±0.4
Stress	-	5.4±1.4 ^c	4.0±1.0 ^f	0.17±0.10 ^g	1.0±0.4 ^g
DIM	15	7.4±2.1 ^b	5.6±1.4 ^b	0.32±0.18 ^b	1.4±0.6
MW-97	100	8.0±2.0 ^b	5.9±1.8 ^b	0.26±0.11 ^b	1.8±0.4 ^b
	25	7.5±3.0 ^b	5.5±2.0 ^b	0.20±0.09	1.8±0.9 ^b

Tab 3. Modulation of MW-97 on serum testosterone concentration in stressed mice. $n = 8$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs stress control. $^dP < 0.05$ vs normal control.

Groups	Dose/mg·kg ⁻¹	Testosterone/ng·L ⁻¹
Normal	-	294 ± 232
Stress	-	58 ± 17 ^a
DIM	15	268 ± 251 ^b
MW-97	100	193 ± 73 ^b
	25	355 ± 102 ^c

Tab 4. Modulation of MW-97 on serum corticosterone concentration in stressed rats. $n = 8$. $\bar{x} \pm s$. $^cP < 0.01$ vs stress control. $^dP < 0.01$ vs normal control.

Groups	Dose/mg·kg ⁻¹	Corticosterone/ μg·L ⁻¹
Normal	-	56 ± 41
Stress	-	197 ± 35 ^f
MW-97	100	120 ± 51 ^c
	25	106 ± 52 ^d

notable difference, while the travelling distance, ambulatory time, and average speed of the stressed mice were all shortened and the resting time was prolonged after treatment with DIM (15 mg/kg). This result suggested that DIM had a sedative effect in stressed mice. The spontaneous motor activity of the mice treated with MW-97 did not show significant change, suggested that MW-97 had no excitatory or sedative effects (Tab 5).

DISCUSSION

Chronically stressed conditions could simulate the symptoms of depression, for instance, chronic stress could induce the atrophy of hippocampus and denaturation of neurons in CA3 region^(5,6). Chronic stress could

induce the lesions or apoptosis of lymphocytes and neutrophils in rats and these changes were mediated by a high concentration of serum corticosterone induced by stress^(7,8). In our study, chronic stress did induce the increase of serum corticosterone and decrease of immune cell counts in mice. MW-97 at the doses of 100 and 25 mg/kg reversed these changes which supported its antistress effects.

It was reported that stress-induced HPA axis hyperaction would inhibit the activity of hypothalamus-pituitary-gonad (HPG) axis. Corticosterone could decrease testosterone synthesis from testicles⁽⁹⁾. Bingama *et al* demonstrated that testosterone could also inhibit the release of corticotropin-releasing factor from hypothalamus and the activity of HPA axis⁽¹⁰⁾. Therefore, the relationship between corticosterone and testosterone was reversing cascade⁽¹¹⁾. The elevation of serum testosterone after treatment with MW-97 maybe related with its inhibition on HPA axis. So, it was suggested that the increasing of serum level of testosterone after MW-97 treatment maybe one of the most important basis for improving of immune activity.

Moreover, this study provided further evidence of the antagonism of MW-97 on chronic stress. The HPA axis of chronically stressed mice was hyperactive and the corticosterone secreted from cortex of adrenal gland was excessive, naturally, the cortex was hyperplastic and plump compensationally. On the contrary, a high concentration of corticosterone could induce medulla pheochromocytes to release catecholamine exhaustively, ultimately, the medulla became atrophic. In fact, the hyperplasia of cortex and atrophy of medulla were all compensational pathological changes induced by stress and they were related with each other, which represented the synergetic effect between HPA axis and sympathetic-adrenal medulla system. Treatment with MW-97 made these two systems balanced and protected adrenal gland

Tab 5. Effect of MW-97 on spontaneous movement in stressed mice. $n = 8$. $\bar{x} \pm s$. $^cP < 0.01$ vs stress control.

Groups	Dose/mg·kg ⁻¹	Distance/cm	Ambulatory time/s	Resting time/s	Speed/cm·s ⁻¹
Normal	-	1906 ± 510	355 ± 53	238 ± 60	5.3 ± 0.9
Stress	-	1805 ± 236	370 ± 30	222 ± 32	4.9 ± 0.4
DIM	15	1188 ± 296 ^c	291 ± 48 ^c	297 ± 48 ^c	4.1 ± 0.5 ^c
MW-97	100	1623 ± 456	341 ± 57	256 ± 57	4.7 ± 0.6
	25	1644 ± 398	354 ± 46	237 ± 48	4.6 ± 0.7

from the pathologic change, this evidence further supported MW-97's antistress and antidepressant effects.

The inulin-type oligosaccharides extracted from tonic medicine Bajitian could improve the stressful condition significantly and did not have excitatory or inhibitory effects on CNS, moreover, it had little toxicity and could be taken orally. All of these results owed inspirative prospect for its using as an antistress agent.

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中药巴戟天寡糖对大、小鼠的抗应激作用

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关键词 巴戟天; 寡糖类; 应激; 睾酮; 皮质酮; 免疫

目的: 探讨巴戟天寡糖(MW-97)对鼠的抗应激效应。
方法: 运用多种非预知性刺激方式以每日一次的频率交替应激雄性大、小鼠 15 d, Vidiomex-V 图象解析系统测定小鼠自发性活动, 常规方法制备肾上腺病理切片, 血细胞分析仪测定血中白细胞水平及其分类, 放免法测定血中应激激素水平。
结果: 慢性应激小鼠肾上腺皮质肥厚, 髓质萎缩甚至消失, 结构层次模糊, 提示肾上腺出现了应激适应性功能衰竭。MW-97 100 mg/kg 处理 15 d, 小鼠肾上腺未出现任何病理改变。此外, 慢性应激小鼠血中白细胞总数及淋巴细胞、单核细胞、中性粒细胞计数都明显降低, MW-97 (25, 100 mg/kg) 翻转这一改变, 使其水平都显著提高。同样, MW-97 也提高慢性应激小鼠血中睾酮水平并降低慢性应激大鼠血中皮质酮水平, 而对其自发性活动没有影响。
结论: MW-97 有抗应激作用, 对中枢神经系统无兴奋或抑制效应, 极有可能成为新一类抗应激药物。

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