Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2015; 4(1): 58-62 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Anti-allergic activity of dichloromethane extract and related lignans from *Magnolia biondii* Pamp.

Yong zhong Zhang*, Xiliang Yang, Song Liu, Xiamin Hu
Medical School, Wuhan University of Science & Technology. Wuhan, 430061 Wuhan, People's Republic of China

***Corresponding author**
Dr. Yongzhong Zhang.
Email: zyzcolin_1228@163.com

Abstract: The objective of present study is to evaluate the effect of anti-allergic activity of dichloromethanolic extract and related lignans from *Magnolia biondii* Pamp. Dried flower buds of *Magnolia biondii* were collected in China, three lignans were isolated from dichloromethane extract of *Magnolia biondii* and identified as: Magnolin, Pinoresinol dimethyl ether, Lirioresinol-B dimethyl ether. Anti-allergic activity of dichloromethanolic extract and related lignans was investigated on histamine-induced contraction of isolated guinea pig ileum, the Schultz-Dale reaction and degranulation of rat mast cells. Histamine-induced contraction of isolated guinea pig ileum was significantly inhibited by pretreatment with CH_2Cl_2 extract（0.2 and 0.1 mg/ml, $P<0.001$）, three lignans have no remarkable effect on contraction of guinea pig ileum. CH_2Cl_2 extract and magnolin exerted inhibitory effect on Schultz-Dale response in ovalbumin-sensitized guinea pig ileum, inhibitory rate were 78.37% (0.2mg/ml) and 45.61% (1.7×10^{-2}mg/ml) respectively. Three lignans have protective effect on rat peritoneal mast cells from degranulation, the effect of magnolin was most powerful. This study demonstrated experimentally that CH_2Cl_2 extract of *Magnolia biondii* Pamp. and related lignans, especially Magnolin possess anti-allergic activity, they may be a valid remedy for allergy.
Keywords: *Magnolia biondii* Pamp., Lignans, Antiallergic, Histamine, Mast cell degranulation.

INTRODUCTION

Dried flower buds of *Magnolia biondii*(Magnoliaceae) ,well known as "Xinyi" in China , have been used for centuries as traditional medicine. This crude drug is used for the prevention and treatment of acute or chronic rhinitis, allergic rhinitis and maxillary sinusitis in China [1]. And now it also has been used for allergic asthma and hay fever[2]. It's often used singly or in combination with other herb medicines. In general, immediate hypersensitivity is mediated by various chemical mediators released from mast cells[3]. Of the preformed and newly synthesized inflammatory substances released on degranlation of mast cells, histamine is the best characterized and most potent vasoactive mediator implicated in anaphylaxis[4]. The main constituents of Xinyi include essential oils (which are composed of cineole, eufenol, chavical methylether, etc.), lignans(incl. Magnolin , Pinoresinol dimethyl ether, Lirioresinol-B dimethyl ether, etc.) and alkaloids (incl. Magnocurarine , etc.). [5-6]. In the past research work, it has been proved that essential oil of Xinyi could inhibit contraction of isolated guinea pig ileum induced by histamine , slow reacting substance of anaphylaxis(SRS-A), as well as the yielding of inflammatory medium, reduce capillary permeability in inflammatory tissue[7]. In addition, it is reported that lignans from Xinyi inhibit the aggregation of rabbit platelet induced by platelet activating factor (PAF)[8].

This paper deals with an evaluation of the effect of CH_2Cl_2 extract and related lignans on histamine-induced contraction of isolated guinea pig ileum, the Schultz-Dale reaction and degranulation of rat mast cells.

MATERIALS AND METHODS

Plant material

Dried flower buds of *Magnolia biondii* Pamp. were collected in Hubei province, China, in February 2009. Professor Wang K.Q. identified the plant material, and the voucher specimen (PA.105) has been deposited at Hubei Academy of Traditional Chinese Medicine and Pharmacy, Wuhan.

Preparation of extract and isolation of lignans

The dried flower buds powder(1.5kg) were defated with petroleum benzine, and then extracted with methylene chloride twice, the extracts were filtered and concentrated in vacuo at 35-40˚C.The w/w yield of the extract was about 4%. 40g extracts were separated with successive silica gel column chromatography and gradually eluted with petroleum benzine - acetidin, Magnolin, Pinoresinol dimethyl ether(PDE), and

Lirioresinol-B dimethyl ether (LDE)were isolated and identified with UV, IR, EIMS, ^{1}HNMR, ^{13}CNMR. Their chemical structures were showed in Fig.1

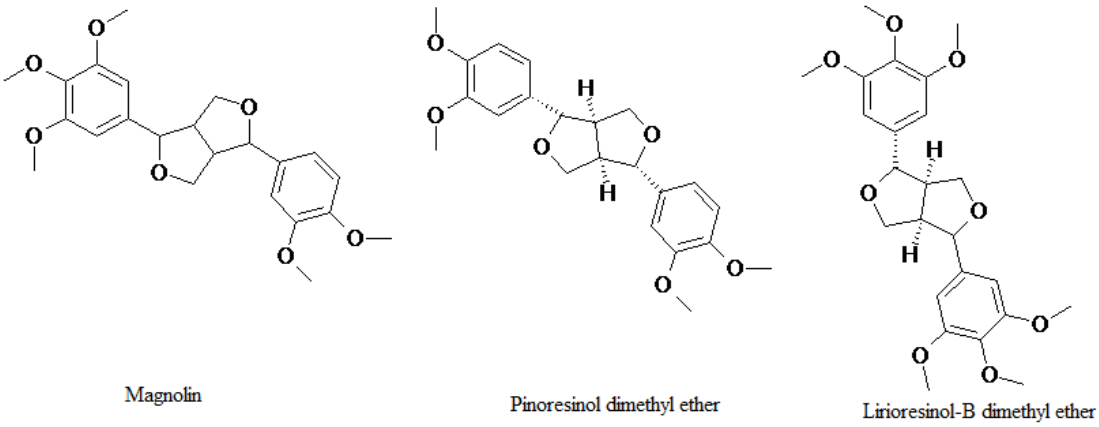

Fig-1: Chemical structures of lignans

The CH_2Cl_2 extract and three lignans were dissolved in 2.5% acacia gum saline solution for pharmacological testing.

Animals

Male Guinea pigs (weighing 180-200g) and Wistar rats (weighing 250-300g) were obtained from Hubei Medical Experiment Animal Center (Wuhan, China), these animals were fed with a standard pellet diet and water *ad libitum*, they were housed in a laminar air flow room maintained at a temperature of 22±1˚C and relative humidity of 55%±10% throughout the study. All experimental designs and procedures had received approval from the Animal Ethics Committee of Wuhan University of Science & Technology.

Chemicals and reagents

Histamine phosphate, ovalbumin were purchased from Sigma Chemical Co., the other chemicals were of analytical grade.

Histamine-induced contraction of Guinea pig ileum (GPI)

Male guinea pigs (180-200g) were sacrificed by a blow to the skull and cervical dislocation and 2 cm pieces of the ileum were dissected from the ileum segment 10-20 cm proximal to the ileocecal valve. Segments were placed in 10 ml organ bath containing Tyrode solution (composition in mM: NaCl 137,KCl 2.7,$CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5;pH 7.4) , bubbled constantly with 95% O_2-5% CO_2 gas and maintained at 37˚C, Tyrode buffer were rinsed every 15 min. The material was mounted at a resting of 1.0g and allowed to equilibrate for 1-2 h. Contractions were recorded with an isotonic transducer (Shanghai Zhongsheng Educate Co.LTD) coupled to a physiological grapher (LMS-2B. Chengdu Instrument Factory,China). Ileum segments were preincubated with saline and various concentrations of CH_2Cl_2 extract and lignans respectively for 2 min, then histamine phosphate (50μg/ ml) was added to the bath, contraction curve were recorded. All experiments were repeated on 5 different preparations obtained from different animals.

Schultz-Dale inhibition test

Schultz-Dale inhibition test was examined as reported previously [9]. Male guinea pigs were sensitized with 5% ovalbumin (OV) dissolved in saline solution, 0.4ml of OV was injected intramuscularly, and 1ml was injected intraperotoneally, 4 weeks later, the sensitized guinea pigs were killed and ileal strips from each animal were mounted in an isolated organ bath using Tyrode solution (37±0.5˚C) .OV(1mg/ml) was added to the bath and responses were recorded for 90s on a physiological grapher, prior to expose to OV, saline and samples were added to the bath for 2 min in order to see the effect of antigen-induced contraction of ileal strips.

Rat peritoneal mast cell preparation and inhibition of mast cell degranulation

Male Wistar rats were sensitized with 5%OV dissolved in 0.9% saline. 0.4ml of OV was injected intramuscularly, and 1ml of OV was injected intraperitoneally. 4 weeks later, the rats were used for experiments. Rat peritoneal mast cells (RPMC) were isolated as previously described [10]. In brief, the rats were killed by bleeding through femoral vein under ether anaesthesia, 20 ml of heparinized Tyrode's solution was injected into the peritoneal cavity of exsanguinated rats . After abdominal massage, the cells in the peritoneal fluid were harvested and separated in 38% bovine serum albumin in glucose-free Tyrode's solution. The cell pellet was washed and suspended in Tyrode's solution. The mast cell count was adjusted to 1-1.5×10^6cells/ml. Cell viability was assessed with the trypan blue exclusion test.

Purified RPMC was resuspended in Tyrode's buffer for the treatment of ovalbumin(10μg/ml).The cells were preincubated in the absence or presence of three lignans and positive medicine disodium cromoglicate (DSCG) at 37˚C for 5 min and then OV(10μg/ml) was added to the cells and incubation proceeded for another 5 min, all the test tubes were taken out of the bath and placed at room temperature for 5 min. More than 97% of the cells were viable as judged by the trypan blue uptake. A drop of mast cells were sucked to the slide from the bottom of tubes, and then the cells were stained with neutral red anhydrous alcohol (0.025%W/V) at 37˚C for 5 min. The numbers of degranulation in 40 mast cells were counted under high power microscope and protection rate from degranulation was calculated using the following equation:

$$\%\text{protection} = (1-a/q)\times 100$$

where a is net degranulation numbers of administration group, q is net degranulation numbers of control.

Statistical analysis

All values are given as mean± standard error of mean for the numbers of experiments noted and statistical analyses were performed using the Student's t-test. Mean differences were considered statistically significant if $P<0.05$.

RESULTS

Effect on contraction of histamine-induced isolated guinea pig ileum

The addition of CH_2Cl_2 extract and three lignans in the organ bath fluid did not modify the resting activity of the preparations. The addition of histamine in the organ bath fluid caused transient contractions on the guinea pig ileum followed by relaxation , this contractile effect was significantly prevented by pretreatment with CH_2Cl_2 extract (0.2 and 0.1 mg/ml, inhibition rate were 70.89% and 42.72%, respectively), three lignans have no remarkable effect on contraction of histamine-induced isolated guinea pig ileum. The results were showed in table 1.

Table-1: Effect of treatment of CH_2Cl_2 extract and lignans of Xinyi on contraction of histamine-induced isolated guinea pig ileum

Group	Dose（mg/ml）	Contraction height(mm)	Inhibition rate (%)
Control	—	16.90±1.38	
CH_2Cl_2 extract	0.2	4.92±1.00 [a]	70.89
	0.1	9.68±2.97 [a]	42.72
Magnolin	1.7×10^{-2}	15.57± 3.12	7.87
	0.85×10^{-2}	15.90±2.56	5.92
PDE	1.5×10^{-2}	16.19±2.89	4.20
	0.75×10^{-2}	16.80± 1.95	0.60
LBDE	1.8×10^{-2}	14.71± 2.23	12.96
	0.9×10^{-2}	15.91± 2.21	5.86

All values are expressed as mean ± S.D., [a]$P<0.01$vs.Control.

Inhibition of Schultz-Dale reaction

The actively sensitized guinea pig smooth muscle showed a typical anaphylactic response when the ileal strip was challenged *in vitro* with the antigen (Schultz-Dale reaction).The control guinea pig ileal strips responded with a large contraction, CH_2Cl_2 extract and Magnolin antagonized ovalbumin caused contraction in sensitized guinea pig ileum *in vitro*. PDE and LBDE had weakly inhibitory effect at the experimental doses. The standard drug DSCG also inhibited the contraction. Results were summarized in table 2.

Table-2: Effect of treatment of CH_2Cl_2 extract and lignans of Xinyi on ovalbumin-sensitized contraction in isolated guinea pig ileum

Group	Dose（mg/ml）	Contraction height(mm)	Inhibition rate (%)
Control	—	12.31±3.5	
CH_2Cl_2 extract	0.2	2.66±0.33[a]	78.37
	0.1	5.87±1.05[a]	52.28
Magnolin	1.7×10^{-2}	6.70±1.23[a]	45.61
	0.85×10^{-2}	7.44±1.15[b]	39.53
PDE	1.5×10^{-2}	8.65±0.67	29.73
	0.75×10^{-2}	10.59±1.21	13.97
LBDE	1.8×10^{-2}	9.71±0.95	21.16
	0.9×10^{-2}	11.07±1.54	10.09

All values are expressed as mean ± S.D., [a]$P<0.01$vs.Control., [b]$P<0.05$ vs. Control.

Effect on sensitised mast cell degranulation

The effect of lignans on sensitised mast cell degranulation was presented in table 3.Three lignans showed significant inhibitory action on degranulation of mast cells caused by the antigen ovalbumin at the concentration of 1mg/ml. Among the lignans, Magnolin showed the most remarkable protection at 1.7mg/ml (P<0.01). DSCG also protected the mast cells from degranulation.

Table-3: Effect of lignans of Xinyi on mast cell degranulation

Group	Dose (mg/ml)	Net degranulation numbers	Protection rate (%)
Control	—	29.75±3.30	
Magnolin	1.7	16.25±4.11 [a]	45.38
	0.17	19.50±4.12[a]	34.45
PDE	1.5	19.25±4.57[a]	35.30
	0.15	26.25±4.86	11.77
LBDE	1.8	18.50±5.26[b]	37.82
	0.18	21.50±4.35[b]	27.73
DSCG	0.01	10.75±2.25[a]	63.87
	0.001	15.50±3.41[a]	47.90

All values are expressed as mean ± S.D., [a]P<0.01vs.Control., [b]P<0.05 vs.Control.

DISCUSSION

This study was carried out to provide scientific basis for the traditional medicinal use of *Magnolia biondii* Pamp., mainly in allergic rhinitis. The CH_2Cl_2 extract of *Magnolia biondii* Pamp. flower bud and three lignans were tested *in vitro* using isolated segments of guinea pig ileal. The present study has showed that the CH_2Cl_2 extract of *Magnolia biondii* Pamp. exerted potent relaxant effect on contraction of guinea pig ileal smooth muscle caused by histamine, which suggested that CH_2Cl_2 extract of *Magnolia biondii* Pamp. had direct inhibitory action on histamine, this activity is similar to that of the essential oil of *Magnolia biondii* Pamp [11]. But three lignans did not block the contraction brought out by histamine, the possible reason is that CH_2Cl_2 extract is a mixture of many components, except for lignans, it also contains some of essential oil.

Schultz-Dale reaction is a kind of I-type allergy mediated by IgE. The sensitized guinea pig ileum releases allergic mediators and induces contraction when challenged with antigen. Inhibition of Schultz-Dale reaction by CH_2Cl_2 extract and Magnolin showed that they might inhibit the release of allergic mediators.

Mast cells participate in many biological responses such as allergic diseases and inflammatory disorders[12]. Mast cell activation causes the process of degranulation that results in releasing of chemical mediators, including histamine, serotonin [13-14], and newly generated compounds produced within minutes of triggering of their IgE receptors, such as leukotrienes [15]. These mediators are considered responsible for immediate hypersensitivity reactions. More recently, mast cells have been identified as a source of several proinflammatory compounds including nitric oxide and cytokines that are produced over longer time periods following activation of the same receptors[16]. In the mast cell study, three lignans significantly inhibited degranulation of mast cells in sensitized rats, suggested the possibility of inhibition of the phenomenon of sensitization, lignans might act by stabilization of the mast cell membrane, thereby preventing its degranulation like the standard drug DSCG, which is not an antihistaminic nor anti-inflammatory nor a bronchodilator. It exerts its action on the mast cell where the decisive effects of allergen antibody reaction are determined.

These *in vitro* test results indicate that CH_2Cl_2 extract exerts antiallergic action by direct antagonism of histamine and by diminishing the release of allergic mediators, the activity of CH_2Cl_2 extract may be due to one or more of the lignans present in the exact, while three lignans have different mechanism for antiallergic action, mainly by diminishing the histamine release from mast cells.

Due to the significant anti-allergic activity exhibited in above models, The CH_2Cl_2 extract of *Magnolia biondii* Pamp. and related lignans, especially Magnolin are responsible for the anti- allergic effect. DSCG, which has been clinically used for allergic diseases, but a lack of absorption through gastrointestinal tract has prevented its use by oral route. Hence, Xinyi and related lignans can be developed as an anti-allergic drug. Further studies are required to investigate the exact anti-allergic effect *in vivo*.

REFERENCES

1. Yang XX, Zhuang ZQ; Advances in chemical and pharmacological studies on Chinese herb Xinyi. Chinese Traditional and Herbal Drugs, 1998; 29:490-2.
2. Li YG, Xu WL, Liu YX, Sun LJ, Guo Y.;Research advance of chemical constituents and pharmacological activities of *Magnolia denudate* Desr. Guangzhou Chemical Industry, 2013; 41:28-9.
3. Wasserman SI, Marquardt DL; Anaphylaxis in Allergy: Principles and Practice 3rd ed. C.V. Mosby : St. Louis; 1988;1365.

4. Petersen LJ, Mosbech H, Skov P; Allergen-induced histamine release in intact human skin *in vivo* assessed by skin microdialysis technique: characterization of factors influencing histamine releasability. J Allergy Clin Immunol ,1996; 97:672-9.
5. Ma YL, Huang QL, Han GQ; A neolignan and lignans from *Magnolia biodii*. Phytochemistry 1996;41:287-8.
6. Fang H, Guo Q, Su W, Deng F, Wang KQ; RP-HPLC determination of Magnolin in Chinese medicine "Xinyi". Chinese J of Pharmaceutical Analysis, 2002; 22:342-5.
7. Wang W, Shen J, Qi Y, Nie H; Experimental Research of Anti-inflammatory Action of Flower Bud of Lily Magnolia. J of Chengdu University of TCM, 2000; 23:58-61.
8. Zhang YZ, Li XL, Guo Q; Inhibitory effect of lignans from *Magnolia biondii* Pamp.on platelet-activating factor-induced platelet aggregation. J of Hubei TCM, 2001;23:7-8.
9. Baruah CC, Gupta PP, Amarnath Late GK, Patnaik, Dhawan BN; Anti-allergic and antianaphylactic activity of Picroliv—a standardised iridoid glycoside fraction of Picrorhiza kurroa. Pharmacological Research 1998;38:487-492.
10. Kanemoto TJ, Kasugai T, Yamatodani A, Ushio H, Mochizuki T, Tohya K, Kitamura Y et al.; Supernormal histamine release and normal cytotoxic activity of Biege rat mast cells with giant granules. Int Arch Allergy Immunol, 1993;100:99-106.
11. Cheng SY, Zhou DX; Antiinflammatory and antiallergic effect of volatile oil of flower bud of *Magnolia denudate* desr.. Chinese J of Modern Applied Pharmacy, 1998;15:7-9.
12. Bochner BS, Schleimer RP; Mast cells, basophils, and eosinophils: distinct but overlapping pathways for recruitment. Immunol. Rev, 2001; 179:5-15.
13. Church MK, Levi-Schaffer F; The human mast cell. J Allergy Clin. Immunol, 1997; 99: 155–160.
14. Alistair TR, Russell IL, Nicole MV; Mast cell function: Regulation of degranulation by serine/threonine phosphatases. Pharmacology & Therapeutics, 2006; 112:425-39.
15. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, Heusser CH, Howarth PH, Holgate ST; Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am. J Respir. Cell Mol. Biol,1994; 10:471-480.
16. Theoharis CT, Konstantinos DA, Asimenia A, Danae AD, Nikolaos S, Bodi Z; Mast cells and inflammation. Biochimica et Biophysica Acta, 2012; 1822:21-33.