8 位被脂肪氨基对称地取代, 显示细胞毒作用最强, 抗癌活性明显提高, 至于它们抗 MDR 肿瘤细胞作用是否也与它们的这种结构有关还有待继续研究。

ROS 是指氧的某些代谢产物和一些反应的含氧产物。生理状态下, 机体产生的自由基与抗氧化防御系统处于相对平衡。在各种病理因子作用下, 机体产生大量自由基, 或机体抗氧化防御系统受到破坏, 造成细胞结构和功能的破坏。本实验结果表明 4 种药物分别与两种细胞共同培养 12、24、48 h, 12 h 即能引起细胞内 ROS 明显增加, 24 h 时细胞内 ROS 的增加达到最大, 曲线明显右移, 48 h 细胞内 ROS 不再显著性增加, 甚至个别出现曲线微弱左移(图略)。同时, 用 DiOC$_6$检测到 4 种药物分别作用两种细胞 12、24 h 后, 线粒体跨膜电位 ($\Delta\Psi_m$) 稍有降低, 至 48 h 后出现 $\Delta\Psi_m$ 明显降低, 这与 Cortassa[9] 等的实验结果相吻合, 表明 ROS 的增加可直接或间接损伤线粒体膜, 造成膜电位下降, 一些学者的研究结果也支持这一结论[9, 10]。

研究发现 ROS 可能作为信号分子介导了细胞对促凋亡信号的反应。因此, 推测这 4 种大黄素蒽醌衍生物抑制 KB 和 KBv200 细胞的增殖, 可能与其通过线粒体途径诱导细胞凋亡有关。因为它们均能增加细胞内 ROS, 当 ROS 增加到一定程度, 即引起细胞脂质过氧化, 从而干扰细胞线粒体的功能使得线粒体 MPTP 开放, 不仅导致跨膜电位崩溃, 也使细胞色素 C 外漏, 最终启动 Caspase 的级联活化从而引起细胞凋亡, 这就是所谓的线粒体依赖性凋亡通路。这 4 种大黄素蒽醌衍生物是否通过该通路发挥抗肿瘤作用, 相关的研究正在进行中。此外, 它们是在大黄素母核结构基础上经过不同基团修饰合成的, 它们对 MDR 细胞无抗药性, 因此对它们构效关系及作用机制关系的进一步研究, 有可能为开发较大黄素作用更强的抗 MDR 肿瘤的药物提供线索。

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Anti-inflammatory activity of ethanol extracts from root of Daphne genkwa

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Abstract: Object To elucidate the anti-inflammatory activity of the ethanol extracts from roots of

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Daphne genkwa Sieb. et Zucc. (EERD). **Methods** The action on acute inflammation was evaluated by the inhibition of histamine-induced vascular permeability, carrageenin-induced rat paw edema and phagocytosis of reticular endothelial system (RES) in mice. The action on chronic inflammation was examined using adjuvant-induced arthritis and cotton pellet-induced granuloma. **Results** EERD produced evident inhibition of vascular permeability and rat paw edema at a dose of 40 mg/kg and enhanced phagocytosis of RES in mice at a dose of 30 mg/kg. Daily treatment at a dose of 30 mg/kg exhibited significant inhibition of granuloma and adjuvant-induced polyarthritis. EERD also inhibited the production of MDA and PGE_2, NO, TNF-α and IL-1β, enhanced the activity of SOD and CAT and reduced the activity of iNOS. **Conclusion** EERD acts as anti-inflammatory agent by mechanisms that involve the inhibition of lipid peroxidation and the release of mediators, the enhancement of activity of SOD and CAT, and the reduction in activity of iNOS as well as the promotion of phagocytosis of RES.

Key words: ethanol extracts from roots of *Daphne genkwa* Sieb. et Zucc. (EERD); anti-inflammatory activity; lipid peroxidation reaction

芫花根乙醇提取物的抗炎活性

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摘 要: **目的** 阐明芫花根乙醇提取物 (EERD) 的抗炎活性。**方法** EERD 对急性炎症的抑制作用采用组胺诱导的小鼠毛细血管通透性增加、角叉菜胶诱导的大鼠足肿胀以及小鼠网状内皮系统 (RES) 对异物的吞噬作用进行分析。对慢性炎症的抑制作用以福氏完全佐剂诱导的多发性关节炎和棉球诱导的大鼠肉芽肿进行分析。**结果** EERD 在剂量为 40 mg/kg 时能明显地抑制小鼠毛细血管通透性增加和抑制大鼠足肿胀; 在剂量为 30 mg/kg 时对 RES 的吞噬能力有提升作用、对大鼠肉芽肿和多发性关节炎表现出显著抑制作用。EERD 也能抑制丙二醛 (MDA)、前列腺素 E_2 (PGE_2)、一氧化氮 (NO)、肿瘤坏死因子-α (TNF-α) 和白细胞介素-1β (IL-1β) 的形成, 增强超氧化物歧化酶 (SOD) 和过氧化氢酶 (CAT) 的活力并钝化诱导型氮氧化物合酶 (iNOS) 的活性。**结论** EERD 的抗炎活性主要是通过抑制脂质过氧化反应和炎症介质的释放、增强 SOD 和 CAT 的活力、钝化 iNOS 的活性以及提升 RES 的吞噬作用实现的。

关键词: 芫花根乙醇提取物; 抗炎活性; 脂质过氧化反应

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1 Introduction

Daphne genkwa Sieb. et Zucc. (Thymelaeaceae) is widely distributed in southern China as well as the regions along the Yellow River[1]. For years, this species has been used as the herbal remedy for treating chronic bronchitis, hepatitis, and arthritis *etc*[1]. A number of studies have disclosed that the roots of *D. genkwa* possess anti-inflammatory, analgesic, anticonvulsant and anti-irritate activity and present evident efficacy in relieving cough and asthma and exhibit remarkable bacteriostatic activity against pneumococcus and opportunistic dermatophytes[1]. The roots of the species have also been used in China as the major component in the prescription for treating rheumatoid arthritis[2]. Despite its medicinal importance, however, this species has not yet been subjected to systematic pharmacological anti-inflammatory evaluation to substantiate its application on therapeutic purposes. Moreover, the mechanisms for anti-inflammatory effect of the species have not been clarified as yet. In an attempt to further confirm the anti-inflammatory activity of the roots of *D. genkwa* and its possible mechanisms of action, the study on inhibition of acute and chronic inflammation by ethanol extracts from roots of *D. genkwa* (EERD) was performed. This paper described *in vivo* efficacy of the extract in inhibiting acute and chronic inflammation of animals and gave possible explanation on mechanisms of action.

2 Materials and methods

2.1 Materials: The roots of *D. genkwa* were collected from southern mountainous region of Anhui Province, China, in November 2002, and authenti-

cated by Prof. YE Ding-jiang, from Faculty of Pharmacy, Nanjing University of Traditional Chinese Medicine, China. A voucher specimen TCM 978 has been preserved in the Herbarium for Faculty of Pharmacy, Nanjing University of Traditional Chinese Medicine, China.

2.2 Animals: Wistar rats weighing 180—220 g were used for the assay of effects on chronic and sub-acute inflammation and KM mice weighing 18—22 g on acute inflammation. The animals were housed at (23±1) ℃, with relative humidity of (55±10)%, 12/12 h light/dark cycle and fed with standard pellet diet and water *ad libitum*. The animals were cared humanly according to the standards for laboratory animals established by People's Republic of China (GB 14923-94, GB 14922-94, and GB/T 14925-94).

2.3 Methods

2.3.1 Preparation of ethanol extracts: The well-pulverized, aerial dried roots of *D. genkwa* (5 kg) were extracted with 3-fold volume of 95% EtOH for seven days at room temperature and further concentrated in vacuum to afford 495 g extracts (accounts for 9.9% of the total material).

2.3.2 Vascular permeability of mice: Mice were randomly classified into three test groups and two control groups (n= 12). The test groups were ig administered EERD emulsion prepared in Tween-80 (Shanghai No. 2 Reagent Plant, China) at doses of 10, 30, and 50 mg/kg, respectively. The control groups ig received saline (blank control) and prednisone acetate (PRE) (Sigma, positive control) at a dose of 30 mg/kg. One hour after EERD treatment, the test mice were sc syringed with histamine (Sigma) at a dose of 1 mg/mL × 200 μL followed by iv injection of 1% Evans Blue (Sigma) at a dose of 100 mg/kg. Twenty minutes later, the tinged area of skin and its content of Evans Blue were assayed as previously described[3].

2.3.3 Carrageenin-induced rat paw edema: Wistar rats were randomly classified into five test groups and two control groups (n= 10). The test groups were ig treated by EERD at doses of 10, 20, 30, 40, and 50 mg/kg, respectively, 30 min prior to injecting of carrageenin (Sigma) (1% × 0.1 mL) into right hind paw of test rats. One hour after inflammation, paw edema of the rats was detected every hour for six consecutive hours as previously described[4]. The other two groups receiving indomethacin (IDT) at a dose of 3.6 mg/kg and saline were used as positive and blank control, respectively.

2.3.4 Contents of MDA and mediators, and activity of enzymes involved in carrageenin-induced inflammation: Wistar rats were randomly divided into five test groups and two control groups (n= 10). The test groups were ig medicated by EERD at doses of 10, 20, 30, 40, and 50 mg/kg, respectively, 30 min prior to injecting 0.1 mL of 1% carrageenin to the hind paw of test rats. Four hours after exposure to carrageenin, the homogenate of edema paw of the rats was prepared for determining the contents of prostaglandin E_2 (PGE_2), madonadialdehyde (MDA), and NO, and the activity of SOD, CAT as well as iNOS according to the procedures described in specifications of reagent kits (Nanjing Jiancheng Biotechnology Institute). Control groups were treated with IDT at a dose of 3.6 mg/kg and saline, respectively. Levels of PGE_2, MDA, and NO were expressed as nmol/mg, while the levels of SOD, CAT, and iNOS were presented in nU/mg.

2.3.5 Freud's complete adjuvant-induced paw edema: Wistar rats were randomly classified into five test groups and two control groups (n= 10). The test groups were ig medicated by EERD at doses of 10, 20, 30, 40, and 50 mg/kg, respectively, one hour prior to injecting 0.1 mL Freund's complete adjuvant (Sigma) to the right hind paw of test rats. The increase in paw edema was recorded every other day for 22 consecutive days to calculate edema rate as previously illustrated[4]. The two control groups were received PRE at a dose of 30 mg/kg (positive control) and saline (blank control), respectively.

2.3.6 Formation of MDA, and mediators, and enzymes involved in adjuvant-induced inflammation: Wistar rats were randomly separated into five

test groups and two control groups (n= 10), each receiving 0.1 mL Freud's complete adjuvat on their hind paw. The five test groups of AA rats were ig treated by EERD at doses of 10, 20, 30, 40, and 50 mg/kg, respectively, for 19 consecutive days. One hour after the last medication, the inflamed paw of the rats was homogenated with PBS in ice bath followed by centrifugation to produce supernatants. The supernatant of homogenate was assayed for the content of IL-1β, TNF-α (TPI Inc. USA), MDA, PGE_2, and NO, and the activity of SOD, CAT, and iNOS according to the procedures described in the specifications of reagent kits. The two control groups of AA rats received IDT at a dose of 3.6 mg/kg (positive) and saline (blank). Levels of PGE_2, MDA, and NO were denoted in mol/mg, and levels of SOD, CAT, and iNOS in nU/mg, while levels of IL-1β, TNF-α in pg/mL.

2.3.7 Cotton pellet-induced granuloma: Wistar rats were randomly divided into five test groups and two control groups (n= 10). The test groups were ig administered EERD at doses of 10, 20, 30, 40, and 50 mg/kg for seven consecutive days, respectively, three days prior to the operating for the formation of granuloma developed by cotton pellet according to the method described by Hajare[5]. The inhibition of the growth of granuloma was assayed using the two control groups receiving IDT at a dose of 3.6 mg/kg and saline, respectively.

2.3.8 Phagocytosis of RES in mice: Mice (♀/♂) were randomly separated into five test groups and two control groups (n= 20). The test groups were orally treated by EERD at doses of 10, 20, 30, 40, and 50 mg/kg for five consecutive days. One hour after the last medication, the test mice were iv syringed with 1% congo-red for determining the expurgation by reticular endothelial system (RES) as previously described[1]. The control groups were ig treated with IDT at a dose of 3.6 mg/kg and saline, respectively.

2.3.9 Statistical analysis: The data acquired in the experiment were processed using Student-Newman-Keuls test software. The results were indicated as $\bar{x} \pm s$.

3 Results

3.1 Effect on vascular permeability in mice: Inflammation is concomitant with the increase of vascular permeability. EERD in used doses exhibited remarkable inhibitory effect on histamine-induced increase of vascular permeability. At a dose of 10 mg/kg, EERD produced evident reduction in Evans Blue permeated from capillary blood vessel by 52.5% and indicated dose-dependent inhibition of up to 68.5%. In contrast to the mice treated with saline, the tinged area by Evans Blue was also reduced significantly and dose-dependently. By comparison, PRE showed similar effect in inhibiting vascular permeability to EERD (Table 1).

Table 1 Effect of EERD on vascular permeability in mice induced by histamine ($\bar{x} \pm s$, n= **12**)

Groups	Dose /(mg·kg^{-1})	Tinged area /mm^2	Evens Blue /(mg·mL^{-1})	Inhibition rate/%
Normal	-	0.02± 0.00	0.00±0.00	-
Saline	-	450.23±35.17$^{\triangle}$	0.62±0.08$^{\triangle}$	-
PRE	30	288.13±29.33***	0.29±0.02*	59.05
EERD	10	319.87±22.89**	0.30±0.01*	52.75
	30	294.19±21.97***	0.29±0.00*	55.90
	50	128.76±17.47***	0.27±0.01*	68.50

$^{\triangle}P$< 0.01 *vs* normal group

$^{*}P$< 0.05 $^{**}P$< 0.01 $^{***}P$< 0.001 *vs* saline group

3.2 Effect on carrageenin-induced paw edema of rats: Theoretically, carrageenin-induced paw edema belongs to sub-acute inflammation. The paw edema of the rats treated by saline rose from 24.8% at one hour to 55.1% at three hours following the exposure to carrageenin and began to scale down from 33.9% at four hours to 30.8% at six hours. In contrast, the paw edema of the rats treated by EERD at used doses did not exceed 18.1% at one hour and 26.7% at three hours. The paw edema of the rats was decreased to less than 20.4% beginning from four hours. In comparison, the EERD at used doses and IDT at dose of 3.6 mg/kg indicated no significant difference in inhibition of carrageenin-induced paw edema (Fig. 1).

3.3 Effects on the generation of MDA, and mediators, and activity of involved enzymes in carrageenin-induced inflammation: EERD in used doses produced positive reduction in generation of MDA, PGE_2, and NO. The inhibition of MDA

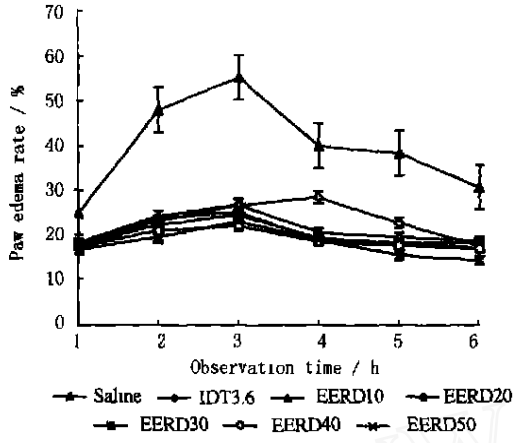


Fig 1 Effect of EERD on carrageenin-induced paw edema of rats ($\bar{x} \pm s$, n = **10**)

generation was observed at a dose of 10 mg/kg ($P < 0.001$) and intensified in response to the dose increase within 30 mg/kg. Further increase in dose treatment (i.e. 40 mg/kg), however, resulted in reduced level of inhibition. Obvious inhibition on the release of PGE_2 and NO was detected at a dose of 10mg/kg ($P < 0.05$) and further enhanced in response to the increased dose-treatment below 40 mg/kg. At a dose of 50 mg/kg, nevertheless, EERD presented reduced inhibition on formation of PGE_2 and NO (Table 2).

With reference to the effects on enzymes involved in inflammation, EERD in used doses revealed prominent increase in the activity of SOD and CAT. Below the dose of 40 mg/kg, EERD produced remarkable enhancement on the activity of SOD, while at a dose of 50 mg/kg, EERD generated less enhancement in the activity of SOD. EERD enhanced the activity of CAT at a dose of 30 mg/kg (Table 3). On the other hand, EERD at a dose of 20 mg/kg indicated positive inhibition on the activity of iNOS and intensified following the increase of dose treatment reaching its maximal inhibition at a dose of 40 mg/kg. Further increase in dose treatment, however, EERD produced less level of inhibition on the iNOS activity (Table 3).

3.4 Effects on adjuvant-induced paw edema of AA rats: The paw edema of AA rats rose from 23.6% at the second day to following the exposure to Freud's complete adjuvant. On the eighth day (the end of latent phase) the edema rose abruptly from 40.3% to 58.9% and then followed by a gradual decrease. EERD in used doses showed considerable inhibitory effects on paw edema of AA rats. The inhibition by EERD began from the fourth day and kept rather steady inhibitive rate until the twentith day. Among the doses used, daily treatment at a dose of 30 mg/kg represented the minimum edema from the beginning of expo-

Table 2 Effect of EERD on carrageenin-induced generation of MDA and mediators ($\bar{x} \pm s$, n= **10**)

Groups	Dose/(mg·kg^{-1})	MDA/(nmol·mg^{-1})	PGE_2/(nmol·mg^{-1})	NO/(nmol·mg^{-1})
Normal	-	0.230±0.04	0.278±0.11	1.39±0.59
Saline	-	0.673±0.09$^{\triangle\triangle}$	0.683±0.12$^{\triangle\triangle}$	1.77±0.58$^{\triangle}$
IDT	3.6	0.263±0.09***	0.459±0.10*	1.13±0.49**
EERD	10	0.462±0.04*	0.367±0.07**	1.49±0.51*
	20	0.383±0.06**	0.375±0.11**	1.31±0.43*
	30	0.237±0.08***	0.329±0.06**	0.85±0.38***
	40	0.449±0.09*	0.289±0.08**	0.79±0.22***
	50	0.633±0.07	0.539±0.09	0.93±0.39***

$^{\triangle}P < 0.05$ $^{\triangle\triangle}P < 0.01$ *vs* normal group; $^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ *vs* saline group

Table 3 Effects of EERD on activity of enzymes involved in carrageenin-induced inflammation ($\bar{x} \pm s$, n= **10**)

Groups	Dose/(mg·kg^{-1})	SOD/(nU·mg^{-1})	CAT/(nU·mg^{-1})	iNOS/(nU·mg^{-1})
Normal	-	32.53±1.67	0.89±0.22	0.152±0.02
Saline	-	25.99±1.07$^{\triangle}$	0.58±0.16$^{\triangle}$	0.292±0.05$^{\triangle\triangle}$
IDT	3.6	30.55±3.02***	0.97±0.25***	0.156±0.03**
EERD	10	30.81±5.14***	0.56±0.13	0.263±0.01
	20	30.65±5.12***	0.58±0.13	0.232±0.01*
	30	31.33±1.23***	0.67±0.12**	0.213±0.02*
	40	30.20±4.28***	0.79±0.13**	0.167±0.02**
	50	28.44±3.71***	0.86±0.08***	0.186±0.03**

$^{\triangle}P < 0.05$ $^{\triangle\triangle}P < 0.01$ *vs* normal group; $^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ *vs* saline group

sure to Freud's complete adjuvant till the end of the experiment. The inhibition on paw edema by PRE, as shown in Fig. 2, was similar to that of EERD at a dose of 10 mg/kg.

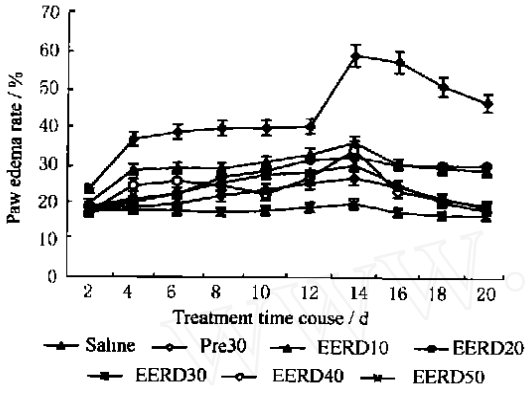


Fig. 2 Effect of EERD on adjuvant-induced paw edema of rats ($\bar{x} \pm s$, n = **10**)

3.5 Effects on adjuvant-induced formation of MDA and mediators, and the activity of involved enzymes in inflamed paw of AA rats: The release of MDA and various mediators including PGE_2, NO, IL-1β and TNF-α is involved in the process of inflammation. EERD in used doses produced obvious inhibition of the generation of MDA and mediators. The inhibition of MDA and PGE_2 was detected at a dose of 10 mg/kg ($P < 0.001$), enhanced at 20 mg/kg and scaled down at a dose of 30 mg/kg. The inhibition of NO, IL-1β and TNF-α was observed at a dose of 10 mg/kg ($P < 0.05$) and enhanced in response to the increase of dose treatment (Table 4). The increase in activity of SOD and CAT implicates the enhanced inhibition of lipid peroxidation. EERD enhanced the activity of SOD and CAT at a dose of 20 and 30 mg/kg, respectively ($P < 0.05$). On the other hand, the reduction of NO is mediated by declining the activity of iNOS. EERD was so observed to inhibit the activity of iNOS at a dose of 20 mg/kg and intensified at 30 mg/kg (Table 5).

Table 4 Effect of EERD on contents of MDA and mediators in adjuvant-induced inflammation tissue ($\bar{x} \pm s$, n = **10**)

Groups	Dose /(mg·kg^{-1})	MDA /(nmol·mg^{-1})	PGE_2 /(nmol·mg^{-1})	NO /(nmol·mg^{-1})	IL-1β /(pg·mL^{-1})	TNF-α /(pg·mL^{-1})
Normal	-	0.23±0.04	0.278±0.109	1.39±0.59	20.37±3.05	186.63±23.40
Saline	-	0.48±0.11$^{\triangle\triangle}$	0.398±0.110$^{\triangle}$	3.18±0.95$^{\triangle\triangle}$	107.39±15.01$^{\triangle\triangle\triangle}$	730.51±63.91$^{\triangle\triangle\triangle}$
IDT	3.6	0.30±0.19*	0.292±0.051**	2.01±0.95**	35.23± 3.38***	299.55±46.32***
EERD	10	0.26±0.09**	0.263±0.050**	2.12±0.78**	51.84± 3.99***	349.94±58.23***
	20	0.21±0.00**	0.252±0.019**	1.48±0.37***	49.19± 5.72***	283.00±34.59***
	30	0.26±0.01**	0.297±0.028*	1.35±0.68***	38.98± 3.40***	209.00±61.34***
	40	0.29±0.05*	0.321±0.017	1.44±0.29*	47.56± 6.82**	248.12±34.89*
	50	0.31±0.04*	0.357±0.043	1.67±0.37*	59.18± 9.73**	297.37±56.14*

$^{\triangle}P < 0.01$ $^{\triangle\triangle}P < 0.001$ $^{\triangle\triangle\triangle}P < 0.0001$ *vs* normal group; $^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ *vs* saline group

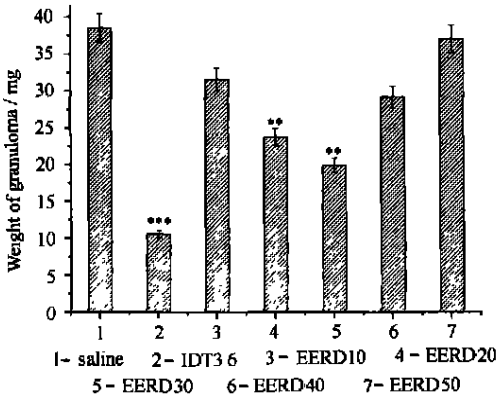
Table 5 Effect of EERD on activity of involved enzymes in adjuvant-induced inflammation tissue ($\bar{x} \pm s$, n = **10**)

Groups	Dose/(mg·kg^{-1})	SOD/(nU·mg^{-1})	CAT/(nU·mg^{-1})	iNOS/(nU·mg^{-1})
Normal	-	32.53±1.67	0.89±0.22	0.15±0.02
Saline	-	21.75±1.14$^{\triangle\triangle}$	0.41±0.19$^{\triangle\triangle}$	0.28±0.09$^{\triangle}$
IDT	3.6	32.38±2.77***	0.69±0.19**	0.20±0.03**
EERD	10	30.87±2.52***	0.81±0.20**	0.21±0.03**
	20	33.57±3.54***	0.86±0.14**	0.17±0.06**
	30	32.19±2.17***	0.92±0.19	0.15±0.09**
	40	29.79±3.56*	0.79±0.10	0.22±0.01**
	50	27.84±4.72*	0.70±0.17	0.26±0.043

$^{\triangle}P < 0.01$ $^{\triangle\triangle}P < 0.001$ *vs* normal group; $^{*}P < 0.05$ $^{**}P < 0.01$ $^{***}P < 0.001$ *vs* saline group

3.6 Effects on development of granuloma in rats: The assay indicated that EERD at a dose of 10 mg/kg slightly reduced the growth of granuloma by 24.3% and gained reduction by 38.56% and 54.03% in response to the increase of dose treatment from 20 to 30 mg/kg, symbolizing its significant inhibition of granuloma formation ($P < 0.01$). Further increase in dose treatment (above 40 mg/kg), however, produced less or no inhibition of granuloma formation (Fig. 3).

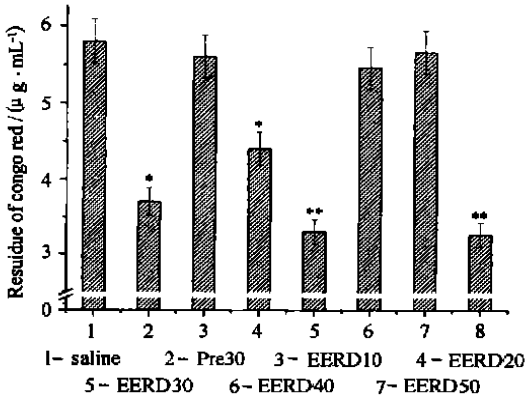
3.7 Effects on phagocytosis of RES: EERD indicated evident enhancement on phagocytosis of RES



** $P < 0.01$ *** $P < 0.001$ *vs* saline group

Fig 3 Effect of EERD on cotton pellet granuloma of rats ($\bar{x} \pm s$, n= **10**)

in test mice. The reduction of congo-red by phagocytosis of RES was observed at a dose of 20 mg/kg ($P < 0.05$) and enhanced at a dose of 30 mg/kg ($P < 0.01$). Further increase in dose treatment, nevertheless, led to gradual decrease in phagocytic activity of RES. In comparison, the capacity for enhancing phagocytosis by IDT was almost the same as that of EERD at a dose of 30 mg/kg (Fig. 4).



* $P < 0.05$ ** $P < 0.01$ *vs* saline group

Fig 4 Effect of EERD on phagocytosis of RES in mice ($\bar{x} \pm s$, n= **10**)

4 Discussion

Inflammation, which involves both innate and adaptive immune mechanisms, is the response of living tissue to cell injury[4]. The results suggested that EERD exhibited considerable inhibition of histamine-induced vascular permeability, carrageenin-induced rat paw edema, adjuvant-induced granuloma, indicating that EERD was an effective agent for the therapy of acute and chronic inflammation.

The process of inflammation is characterized by the release of mediators including histamine[3], prostaglandins (PGs), NO[6], IL-β and TNF-α[7] and by lipid peroxidation that results in the production of MDA[8]. Histamine is released by mast cell through degranulation and causes vasodilation through combining histamine receptor on vascular endothelial cells[9]. PGE_2 is the metabolite of arachidonic acid catalyzed by cyclooxygenase during the tissue injury[10]. It has been reported that small fluxes of NO produced by iNOS contributes to the immune defense against invading microorganisms and tumor cells. However, excessive generation of NO is associated with septic shock, acute and chronic inflammation, autoimmune diseases and arteriosclerosis[11]. NO has also been evidenced to be a stimulant radical for the release of PGE_2[12]. IL-1 and TNF-α are released mainly by activated monocytes and macrophages during acute inflammation and termed as proinflammatory mediators[13]. Lipid peroxidation is triggered by free oxygen radicals in a well-oxygenated environment leading to the production of MDA[14]. Under physiological conditions, the production of inflammatory mediators and free radicals are abolished by SOD and CAT[15]. The considerable inhibition of histamine-induced vascular permeability and formation of PGE_2 implies that EERD may act as antagonist of histamine receptors and the inhibitor of cyclooxygenase. The reduction in NO formation and inhibition of activity of iNOS indicates that EERD is also the possible iNOS inhibitor. The release of IL-1 and TNF-α by monocytes and macrophages needs activation and initiation. Obvious inhibition on release of IL-1 and TNF-α suggests that proper dose of EERD might function as the inhibitor for activation monocytes and macrophages. Prominent reduction by EERD in the content of MDA in inflamed tissues further confirms the quenching and scavenging effect on free radicals. RES is a part of host defense systems against invading microorganisms by phagocytosis. Phagocytosis is the process by which cells bind and internalize relatively large particles containing dead cell

or cell debris induced by complementary cascade reaction[1] or by mediators through apoptosis[16]. The striking enhancement for clearing Congo-red in the bloodstream of mice by EERD symbolizes its opsonic effect on the phagoytosis, which is definitely helpful in fatcilitating rehabilitation of injured tissues.

Therefore, the anti-inflammatory effect of EERD was realized, at least in part, by inhibiting lipid peroxidation, release of mediators and enhancing the activity of SOD and CAT as well as reducing the activity of iNOS and potentiating the phagocytosis of RES.

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乌苏里藜芦碱对血小板聚集及凝血与出血时间的影响

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摘 要: **目的** 研究乌苏里藜芦碱 (VnA) 对大鼠的抗血小板作用及其对小鼠凝血时间和出血时间的影响。**方法** 比浊法测定正常大鼠及血瘀模型大鼠血小板聚集百分率, 观察 VnA 抗血小板作用。毛细玻璃管法测定小鼠全血凝血时间 (CT); 比较等效抗凝剂量的 VnA 及肝素对小鼠尾出血时间 (BT) 的影响。**结果** VnA (45、30、15 μg/kg, iv) 对 ADP 诱发的大鼠血小板聚集有明显抑制作用, 且呈剂量依赖性。VnA (12.5、25、50、100 μg/kg, ip) 可明显延长小鼠 CT 和 BT, 等效抗凝剂量的 VnA (49.3 μg/kg, ip) 所致 BT 延长略低于肝素 (1.25 mg/kg, ip), 但无统计学意义。**结论** VnA 具有显著抗血小板作用, 能显著延长 CT, 对 BT 的延长作用不超过肝素。

关键词: 乌苏里藜芦碱; 抗血小板作用; 凝血时间; 出血时间

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