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5味中药对鸡白痢沙门氏菌的体外联合抑菌研究

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[摘要] 【目的】探讨诃子、虎杖、紫花地丁、夏枯草和何首乌提取物对鸡白痢沙门氏菌的抑制效果。【方法】采用超声波处理、乙醇回流、水浴加热的方法,提取诃子、虎杖、紫花地丁、夏枯草、何首乌5味中药的有效成分,采用二倍试管稀释法分别测定各中药提取物对鸡白痢沙门氏菌的最小抑菌质量浓度(Minimal inhibitory concentration, MIC)和最小杀菌质量浓度(Minimal bactericidal concentration, MBC),并采用肉汤稀释棋盘法,测定中药提取物对鸡白痢沙门氏菌的联合抑菌效果。【结果】诃子、虎杖、紫花地丁、夏枯草、何首乌提取物对鸡白痢沙门氏菌的MIC、MBC值分别为15.60~62.50,15.60~125.00 mg/mL(以生药计算)。虎杖、夏枯草、紫花地丁、何首乌与诃子联合用药时,对鸡白痢沙门氏菌的联合抑菌指数(FICI)均为0.25;虎杖与紫花地丁、夏枯草,紫花地丁与夏枯草联合用药时的FICI为1.25~2;何首乌与虎杖、紫花地丁、夏枯草联合用药时的FICI均大于2。【结论】虎杖、夏枯草和诃子提取物对鸡白痢沙门氏菌具有较强的体外抑菌活性。诃子与虎杖、紫花地丁、何首乌、夏枯草联合应用,其抑菌呈协同作用;虎杖与紫花地丁、夏枯草,紫花地丁与夏枯草联合应用,其抑菌呈无关作用;何首乌与虎杖、紫花地丁、夏枯草联合应用,其抑菌呈拮抗作用。

[关键词] 中药提取物;鸡白痢沙门氏菌;体外抑菌;联合抑菌

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Combined anti-bacterial effects of five Chinese traditional medicines against *Salmonella pullorum*

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Abstract: 【Objective】The combined antibacterial effects of extracts from 5 Chinese traditional medicines including *Terminalia chebula* Retz, *Polygonum cuspidatum*, *Viola philipica*, *Prunella vulgaris*, and *Polygonum multiflorum* against *Salmonella pullorum* were studied. 【Method】Ultrasonic processing, ethanol refluxing and water bath heating methods were used to extract the effective components of *Terminalia chebula* Retz, *Polygonum cuspidatum*, *Viola philipica*, *Prunella vulgaris*, and *Polygonum multiflorum*. Double tube dilution method was used to determine the minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of the extracts against *Salmonella pullorum*. Then broth chess-

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board method was used to determine the combined effect of extracts from traditional Chinese medicines on *Salmonella pullorum*. 【Result】 The MIC and MBC ranges of the extracts of *Terminalia chebula* Retz, *Polygonum cuspidatum*, *Viola philipica*, *Prunella vulgaris*, and *Polygonum multiflorium* against *Salmonella pullorum* were 15.60–62.50 and 15.60–125.00 mg/mL (according to crude drugs), respectively. The FICI values of effective components of *Terminalia chebula* Retz combined with *Polygonum cuspidatum*, *Viola philipica*, *Prunella vulgaris*, and *Polygonum multiflorium* were all 0.25. The FICI values of effective components of *Polygonum cuspidatum* combined with *Viola philipica*, *Prunella vulgaris*, *Viola philipica* combined with *Prunella vulgaris* were 1.25–2. The FICI values of effective components of *Polygonum multiflorium* combined with *Polygonum cuspidatum*, *Viola philipica* and *Prunella vulgaris* were greater than 2. 【Conclusion】 The extracts of *Polygonum cuspidatum* and *Terminalia chebula* Retz had strong effects against *Salmonella pullorum* *in vitro*. *Terminalia chebula* Retz combined with *Polygonum cuspidatum*, *Viola philipica*, *Prunella vulgaris* and *Polygonum multiflorium* had synergistic effects. *Polygonum cuspidatum* combined with *Viola philipica* and *Prunella vulgaris* had no synergistic effects. *Polygonum multiflorium* combined with *Terminalia chebula* Retz, *Polygonum cuspidatum*, *Viola philipica* and *Prunella vulgaris* had antagonism effects.

Key words: herbal extract; *Salmonella pullorum*; antibacterial activity *in vitro*; combination antibacterial

鸡白痢杆菌病是由沙门氏菌引起的一种多发和常发传染病,对雏鸡危害较严重,主要侵害2~3周龄内的雏鸡,世界各国都有发生,造成的经济损失惨重。该病对育成鸡和成年鸡呈慢性或隐性感染,特别是病愈后1个月的鸡,主要损害生殖系统,影响生产性能,病愈鸡可长期带菌和经蛋传播感染,成为危险传染源,而且鸡白痢沙门氏菌能够在家禽肠道内定殖,在加工家禽胴体时,污染鸡肉,继而进入食物链,成为人类沙门氏菌感染的潜在来源^[1-3]。而兽药残留于动物机体内,在肌肉、内脏及鸡蛋中药物残留超标,休药期较短等现象经常有发生。据报道,人体在长期低剂量抗生素的胁迫下,体内的耐药菌株增加,耐药菌株可能产生超级耐药性,对人类的健康产生严重的威胁^[4-5]。随着人们对健康关注度的增加,在家禽生产中,绿色、无公害、无耐药性的新抗菌中兽药将逐步成为市场的主导。为寻找抗鸡白痢沙门氏菌的中兽药,本试验研究了诃子、虎杖、紫花地丁、夏枯草、何首乌等5味中药对鸡白痢沙门氏菌的联合抑菌作用,筛选出联合抑菌增强的中药,为研发抗鸡白痢沙门氏菌的中兽药奠定基础。

1 材料与方法

1.1 材 料

1.1.1 菌种与培养基 鸡白痢沙门氏菌(CVCC1792)菌种,购自中国兽医药品监察所。MH肉汤培养基(Meller-Hinton broth)、营养琼脂培养基(Nutrient agar)、麦康凯培养基(MacConkey

agar)、Baird Parker 琼脂基础培养基(Baird-Parker agarbase)、SS 琼脂培养基(Salmomella Shigella agar),均购自北京奥博星生物技术有限公司。

1.1.2 主要仪器 包括中草药粉碎机(天津市泰斯特仪器有限公司)、手提式高压灭菌器 YWQ-SG41-280(海益恒试验仪器有限公司)、电热恒温培养箱 PYX-DHS-40X-50(上海跃进医疗器械厂)、SW-CJ-IB型超净工作台(苏州净化设备有限公司)、旋转蒸发表仪 R502B(上海亚荣生化仪器厂)、HS-120D 超声波清洗机(上海新芝生物技术研究所)。

1.1.3 中药材 虎杖(产地四川,批次 081102)、诃子(产地云南,批次 090401)、紫花地丁(产地四川,批次 110502)均购于四川雅安惠民堂药业连锁有限责任公司;何首乌(产地宜宾,批次 080933)购于四川宜宾市中药材基地;夏枯草(产地四川,批次 112403)购于成都市荷花池中药材市场。

1.2 方 法

1.2.1 中药提取物的制备 参考文献[6-10]的方法,采用超声波处理、乙醇回流、水浴加热方法制备5味中药的提取物,最后将提取物浓缩得到的提取物浸膏,编号后置于4℃冰箱中保存。

1.2.2 鸡白痢沙门氏菌悬液的制备 取9支灭菌试管,每支试管中加入4.5 mL 生理盐水,第1管加入0.5 mL 在MH肉汤培养基上培养的鸡白痢沙门氏菌,之后依次10倍稀释至第9管。用微量移液器取10⁵~10⁹稀释梯度的稀释液0.1 mL于营养琼脂培养基上,用“L”棒均匀涂布,每个稀释度涂3个

平板,37 ℃培养24 h后,对菌落在30~300个的平板进行计数,求平均值,以CFU/mL为单位。最后将其稀释至 $10^6\sim10^7$ CFU/mL菌落。

1.2.3 中药提取物对鸡白痢沙门氏菌的最小抑菌质量浓度(MIC)、最小杀菌质量浓度(MBC)测定

采用二倍试管稀释法测定:取菌液100 μL分别加入含有不同质量浓度(以生药计算)中药提取物的MH肉汤培养基中,肉汤培养基中中药提取物质量浓度自第1试管开始依次倍比稀释至第7试管(最终质量浓度分别为250,125,62.5,31.25,15.63,7.81和3.95 mg/mL),以第8试管中不加中药提取液为空白对照。各试管中MH肉汤培养基和药液的量均为1 mL,37 ℃培养24 h后取出,观察细菌生长情况,与空白对照比较,以肉眼观察无细菌生长的最低浓度判定为中药的最小抑菌质量浓度。

将上述中药组完全抑制的各管中液体涂布接种于营养琼脂培养基平板中,于37 ℃培养24 h后观察结果。以肉眼观察菌落不生长视为细菌被100%杀灭,此时的最低药物质量浓度值为其MBC值。参考刘忠义等^[11]介绍的判断标准,按照试管中细菌的生长情况判定,当MIC<7.80 mg/mL,为高度敏感;7.80 mg/mL≤MIC≤250 mg/mL,为中度敏感;MIC>250 mg/mL,为不敏感。

1.2.4 5味中药联合抑菌试验

采用肉汤稀释棋盘法^[12],测定(3个重复)5味中药联合作用时的体外抗菌活性,以各药对应MIC值的2倍、1倍、1/2倍、1/4倍、1/8倍等浓度分别进行联合药敏试验,重复3次。试验结果以联合抑菌指数(FICI)作为联合

药敏试验的判断依据:FICI≤0.5为协同作用;0.5<FICI≤1为相加作用;1<FICI≤2为无关作用;FICI>2为拮抗作用^[13]。

2 结果与分析

2.1 中药提取物对鸡白痢沙门氏菌的体外抑菌活性

5味中药提取物对鸡白痢沙门氏菌的体外抑菌活性见表1。由表1可知,不同中药提取物对鸡白痢沙门氏菌的抑菌效果不同,其中虎杖提取物对鸡白痢沙门氏菌的MIC和MBC值均为15.60 mg/mL;夏枯草、诃子提取物的MIC和MBC均为31.25 mg/mL;何首乌、紫花地丁提取物的MIC值为62.50 mg/mL,MBC值为分别为62.50和125.00 mg/mL。

表1 5味中药提取物对鸡白痢沙门氏菌的抑菌活性

Table 1 Antimicrobial activity of active ingredients in extracts of five tradition Chinese medicines against *Salmonella pullorum*

药物 Medicine	MIC	MBC
虎杖 <i>Polygonum cuspidatum</i>	15.60	15.60
夏枯草 <i>Prunella vulgaris</i>	31.25	31.25
诃子 <i>Terminalia chebula</i> Retz	31.25	31.25
紫花地丁 <i>Viola philipica</i>	62.50	125.00
何首乌 <i>Polygonum multiflorium</i>	62.50	62.50

2.2 中药提取物对鸡白痢沙门氏菌的体外联合抑菌活性

5味中药提取物对鸡白痢沙门氏菌的体外联合抑菌活性见表2。

表2 5味中药提取物对鸡白痢沙门氏菌的联合抑菌活性

Table 2 Combined antimicrobial effects of active ingredients in extracts of five tradition Chinese medicines against *Salmonella pullorum*

联合用药 United drug	MIC 联合/(mg·mL ⁻¹) MIC united	FICI	联合用药 United drug	MIC 联合/(mg·mL ⁻¹) MIC united	FICI
A-B	A:15.60	1.25	B-D	B:62.50	2
	B:15.60			D:31.25	
A-C	A:15.60	>2	B-E	B:7.80	0.25
	C:62.50			E:3.90	
A-D	A:15.60	2	C-D	C:31.25	>2
	D:31.25			D:62.50	
A-E	A:1.95	0.25	C-E	C:3.90	0.25
	E:3.90			E:3.90	
B-C	B:31.25	>2	D-E	D:3.90	0.25
	C:62.50			E:3.90	

注:A.虎杖;B.紫花地丁;C.何首乌;D.夏枯草;E.诃子。当FICI≤0.5时为协同作用;0.5<FICI≤1时为相加作用;1<FICI≤2时为无关作用;当FICI>2时为拮抗作用。

Note: A. *Polygonum cuspidatum*; B. *Viola philipica*; C. *Polygonum multiflorium*; D. *Prunella vulgaris*; E. *Terminalia chebula* Retz.
FICI≤0.5, synergies effect; 0.5<FICI≤1, additive effect; 1<FICI≤2, independent effect; FICI>2, antagonism effect.

由表2可知,虎杖(A)、紫花地丁(B)、何首乌(C)、夏枯草(D)与诃子(E)联合用药对鸡白痢沙门氏菌的FICI值均为0.25,表现为协同作用。其中紫花地丁提取物的MIC从62.50降到7.80 mg/mL,诃子提取物的MIC值从31.25降到3.90 mg/mL。虎杖与紫花地丁、虎杖与夏枯草、紫花地丁与夏枯草对鸡白痢沙门氏菌的FICI值为1.25~2,表现为无关作用。虎杖与何首乌、紫花地丁与何首乌、何首乌与夏枯草对鸡白痢沙门氏菌的FICI均值大于2,表现为拮抗作用。

3 讨 论

不同中药提取物的抑菌活性不同,大多数中药的有效提取物对细菌、病毒等都有一定的作用。本研究结果表明,虎杖、诃子、紫花地丁、夏枯草、何首乌等5味中药有效成分对鸡白痢沙门氏菌均有一定的抑杀效果。虎杖具有广谱的抗菌活性,对金黄色葡萄球菌、甲型或乙型链球菌、大肠杆菌、伤寒杆菌、肺炎双球菌以及绿脓杆菌等均有抑制作用^[14-15]。本试验研究发现,虎杖提取物对鸡白痢沙门氏菌的体外抑菌效果明显,其MIC值为15.60 mg/mL,说明虎杖对细菌的抗菌活性显著。陈虹等^[16]研究报道,诃子单味药对鸡白痢沙门氏菌的MIC为31.25 mg/mL,与本试验研究结果相符,其抗菌活性成分可能为诃子多元酚类,但具体是何种成分发挥作用还有待进一步研究。据李安林等^[17]研究表明,夏枯草中总黄酮有抗菌作用,但尚未见报道其对鸡白痢沙门氏菌的抑菌作用。本研究结果表明,夏枯草提取物对鸡白痢沙门氏菌具有抗菌效果。

本研究结果表明,诃子与虎杖、紫花地丁、何首乌和夏枯草联合用药对鸡白痢沙门氏菌的FICI均为0.25,表现为协同作用,联合用药后诃子提取物MIC值为3.90 mg/mL,而虎杖、紫花地丁、何首乌和夏枯草的4味中药提取物MIC值为1.95~7.80 mg/mL,诃子和其他4味中药提取物的MIC值都降低,说明这4味中药与诃子联合用药后抑菌活性明显增强,该结果可能与每味中药的提取方法有关。

本研究中,虎杖和紫花地丁联合用药的FICI为1.25,表现为无关作用。但是紫花地丁提取物MIC值由62.50 mg/mL降为15.60 mg/mL,可知虎杖提取物增强了紫花地丁有效成分的作用,目前尚未见这2种中药联合抑菌方面的报道,为虎杖和紫花地丁药效的进一步研究奠定了理论基础。

本研究中,何首乌和虎杖、紫花地丁、夏枯草联

合用药的FICI均大于2,表现为拮抗作用。何首乌提取的有效成分是二苯乙烯苷,虎杖提取的有效成分是虎杖苷,紫花地丁和夏枯草提取的有效成分是总黄酮,当何首乌与虎杖、紫花地丁、夏枯草联合用药时,提取的有效成分可能发生了化学反应,有效成分不能发挥其抗菌作用,因而表现为拮抗作用。

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