小鼠腹泻模型的制备与腹泻指数的应用

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摘要 介绍用番泻叶复制小鼠腹泻模型的规范操作。以稀便率与稀便级的乘积表示的腹泻指数作为主要观察指标，它可以全面反映个体动物的腹泻程度并具有可比性。结果表明：小鼠的腹泻指数呈正态分布；各造型组组间的腹泻指数无差异；同组小鼠连续6d造型其腹泻指数也无差异，表现出良好的重现性。该模型可推荐作为筛选抗腹泻药物使用。

关键词 药理模型 腹泻 腹泻指数

为了研究抗腹泻药物，国内外许多学者对动物腹泻模型进行了大量的研究。但迄今为止，模型的观察指标还不够全面，不够统一，有的指标例如腹泻率，它只能反映群体动物的腹泻比例，却不能反映个体动物的腹泻程度。有的文献[1]采用"稀便率"与"稀便级"来衡量腹泻程度，但这2个指标只能各自单独表示腹泻的一个方面，当2个指标出现相反的结果时就难以作出准确的判断，本文除对复制小鼠腹泻模型的规范操作进行探讨外，进而提出用"腹泻指数"这一指标来对腹泻程度进行全面的综合评价。

1 材料

动物：昆明种小鼠，雌雄兼用，体重20～25g，由本校动物房提供。

造型剂：番泻叶煎剂。浓度0.3g（生药）/ml。番泻叶经本校标本馆鉴定为狭叶番泻 *Cassia angustifolio* Vahl的小叶。定性滤纸：102中速，新华造纸厂。盐酸黄连素片：南京第二制药厂，批号：900704

2 方法与结果

2.1 腹泻模型的建立

2.1.1 造型剂的制备：取番泻叶加水煮沸约10min二层纱布过滤，滤液减压浓缩成0.3g/ml冰箱保存，用时水浴加温至25℃。

2.1.2 分组与造型：取已在实验室饲养2d的健康小鼠，随机分为对照组及若干造型组，每天分别灌胃自来水0.5ml、番泻叶煎剂0.5ml，连续若干天。小鼠单只单笼饲养，鼠笼笼底垫有滤纸及铁丝网。造型前10h禁食，自由饮水。

2.1.3 观察指标：参考文献[1]并作改进。①腹泻率：一组动物中排稀便的动物数与该组动物总数之百分比。②稀便率：每只动物所排的稀便数与总便数之比。③稀便级：表示稀便的程度。以稀便污染滤纸形成污迹面积的大小定级。分为4级，标准如下：

级数	1	2	3	4
污迹直径（cm）	<1	1～1.9	2～3	>3

统计时先逐个统个每一堆稀便的级数，然后将该鼠所有稀便级数相加除以稀便次数得稀便的平均级数，简称稀便级。④腹泻指数：稀便率与稀便级的乘积。

2.1.4 判断标准：①造型成功的标准：造型组与对照组比较，腹泻率及腹泻指数差异。②治疗有效的标准，给药组与对照组比较或给药组给药前后比较，腹泻指数降低等有统计学意义。

2.1.5 结果：用2批小鼠（每批11只）连续6d造型，腹泻率均为100%，腹泻指数（$\bar{x}\pm SD$）

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分别为1.07±0.16，0.99±0.10，而对照组（n＝11)2项指标均为0。

2.2 稀便率、稀便级与腹泻指数的比较：取小鼠30只，随机分为3组，按规定造型，结果见表1。稀便率与稀便级均有组间差异，而腹泻指数却无差异。

2.3 腹泻指数的统计化学分布规律：取雄性小鼠12只，雌性小鼠10只分别连续造型10d，结果见表2。经统计学"数据有无偏态的处理"[2]显示：无论雄或雌小鼠每天每组的腹泻指数呈正态分布；每只小鼠连续10d的腹泻指数呈正态分布。

表1 小鼠稀便率、稀便级和腹泻指数的比较（ x̄±SD ）

组别	动物数	稀便率	稀便级	腹泻指数
1	10	0.23±0.08	2.03±0.42	0.47±0.21
2	10	0.44±0.09***	1.25±0.31***	0.55±0.23
3	10	0.36±0.11***	1.61±0.35***	0.58±0.39

P<0.01 （与第一组比，t检验）

2.4 腹泻指数的差异性及重现性考察

表2 不同性别小鼠连续造型10d的腹泻指数（ x̄±SD ）

性别	动物数	天 数 （d） 1	2	3	4	5
雌	10	0.76±0.25	0.90±0.31	0.62±0.29	0.61±0.35	0.83±0.30
雄	12	0.78±0.38	0.76±0.34	0.73±0.30	0.74±0.28	0.77±0.43

性别	动物数	天 数 （d） 6	7	8	9	10
雌	10	0.88±0.26	0.86±0.42	0.87±0.30	0.88±0.28	0.86±0.23
雄	12	0.87±0.81	0.83±0.40	0.93±0.35	0.92±0.22	0.91±0.18

2.4.1 组间差异性比较：将小鼠随机分为1组对照组及7组造型组，按规定造型，结果见表3 t检验结果：每一批造型组组间的腹泻指数均无差异。

表3 二批小鼠造型、各组的腹泻指数比较（ x̄±SD ）

	组别	1	2	3	4	5	6	7	对照
第一批	动物数	12	12	11	11	12	11	12	11
	腹泻指数	1.10±0.28	1.09±0.29	1.16±0.24	1.12±0.41	1.07±0.35	1.13±0.31	0.98±0.31	0
第二批	动物数	12	12	12	12	12	11	11	11
	腹泻指数	1.18±0.37	1.08±0.23	1.17±0.30	1.12±0.29	1.18±0.32	1.03±0.21	1.13±0.27	0

2.4.2 同组动物连续6d造型，腹泻指数重现性比较：取小鼠30只随机分为3组，一组为对照组，另2组分别灌胃0.33ml及0.5ml造型剂，连续6d造型，结果见表4。2种剂量组每天的腹泻指数均无差异。

表4 2种剂量连续造型6d的腹泻指数（ x̄±SD ）

剂量（g/只）	天数（d）	1	2	3	4	5	6
0.1	n＝10	0.46±0.14	0.61±0.43	0.55±0.28	0.51±0.12	0.59±0.44	0.58±0.36
0.15	n＝10	0.63±0.16	0.78±0.28	0.70±0.22	0.73±0.24	0.62±0.39	0.71±0.21
对照	n＝10	0	0	0	0	0	0

2.5 验证试验：盐酸黄连素片对番泻叶所致腹泻小鼠的治疗作用。将盐酸黄连素片制成4.5mg/ml的水悬液，每天灌胃 2.25mg/只，对照组灌胃0.5ml自来水，结果见表5。治疗后的腹泻指数与治疗前相比，与对照组相比均有显著差异，判为治疗有效。

3 小结与讨论

3.1 为了使统计指标时减少误差，需作几条规定。①粪便次数：以每粒或每堆（ 不能分

肺组织病变减轻，红芪对照组PaO_2也有所升高，表明红芪能改善低氧血症，纠正呼吸性酸中毒，从而对红芪治疗呼吸窘迫综合征提供了理论依据。

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清粒数者）为一次。②干便与稀便的区分以滤纸上有无污迹为标准。③级数直径的测量：圆型者测其直径；椭圆型或不规则形状测其最长和近似圆的直径，两数相加除以2。④观 察 时间：给造型剂后的5h内。

3.2　注意事项：垫纸应选用滤纸，不宜用卫生纸及废报纸。在实验中须防止小鼠咬纸防止小鼠粪便集中成堆。

3.3　采用本剂量造型，其腹泻潜伏期约为1.77±0.42h。

3.4　曾试以粪便重量作为腹泻指标，但此法费时费劲，误差较大，后被淘汰。

3.5　曾用生大黄造型，但其煎剂的制备影响因素多，对小鼠体质损伤大，又易形 成 便秘，而番泻叶没有以上弊病，同时用量也小，故选用番泻叶。

3.6　用本法造型番泻叶煎剂的液温对腹泻指数的影响较大，作者将另文讨论。

3.7　在腹泻模型中应用腹泻指数这一指标来反映稀便的程度，它既可考虑到稀便量的变化，又能考虑到质的因素，比单用稀便率与稀便级这2个指标更全面更客观，并使之具有可比性，统计学又证实，小鼠的腹泻指数呈正态分布，可按参数统计处理。试验又显示小鼠的腹泻指数组间无差异并具有良好的重现性。

3.8　用本法复制小鼠腹泻模型具有操作简单，设备简易，周期短等特点，本文所提出的主要观察指标符合中西医学腹泻病中有关"证"和症状的概念，同时又被验证实验所证实，作者认为，该模型可以推荐作为筛选抗腹泻药物之用。

表5　盐酸黄连素对番泻叶所致腹泻小鼠的治疗作用（$\bar{x} \pm SD$）

	动物数	对照组 9	治疗组 19
治疗前(造型第2天)	腹泻率(%)	100	100
	腹泻指数	1.05±0.38	0.83±0.24
治疗后(给药第3天)	腹泻率(%)	100	89.5
	腹泻指数	0.92±0.35	0.59±0.30** △△

治疗后与对照组比：** $P<0.05$
治疗前后相比：△△ $P<0.05$

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the content of baicalin in this preparation. As a result of stability test, the shelf life at 25°C is estimated to be 1.97 years.

Studies on Effective Compositions of Pinecone Ⅱ. Determination of Polysaccharides in Cone of Chinese Pine (*Pinus tabulaeformis*)

Li Haozhi, Lu Yongjun, Bai Gang, et al

Quantitative determination of polysaccharides in pine cones by phenol-sulfuric acid method was studied, and the effects of concentration of phenol-sulfuric acid and reaction temperature on color formation were investigated. It was found that the absorbance is linearly correlated to polysaccharide concentrations between $10 \sim 78 \mu g/ml$ ($r = 0.9999$). The analytical recovery was 99.8%, CV% was 1.2% and the minimal detectable concentration was $5\mu g/ml$.

Artificial Neural Network Method for Quality Estimation of Traditional Chinese Medicine

Cai Yudong, Gong Jiawen, Cheng Zhaonian, et al

An artificial neural network method for quality estimation of traditional Chinese medicine was suggested, and quality of Hou-Po was estimated by the proposed method in comparison with the analytical results of gas-liquid chromatography. The successful rate reached 100%. The results showed that the neural network method is reliable, and therefore may be referred to as an effective technique for the quality estimation of traditional Chinese medicine.

Protective Effect of Paeonol Against Ischemia Reperfusion Damage in Cardiac Mitochondria Membrane of Rats

Zhang Weiguo and Zhang Zhishan

60mg/kg·d ip, of paeonol were given to rats for 15 days. The myocardial ischemia reperfusion injury model was produced by occluding the left coronary artery and releasing the occlusion in rats. This significantly decreased myocardial Ca^{++}-ATPase activities and CH/PL radical, FFA content, improved mitochondrial membrane fluidity and kept them away from oxygen free radical damage. These results indicated that paeonol has membrane protective effect on myocardial ischemia reperfusion injury probably by inhibiting the oxygen free radicals and subsequent lipid peroxidation.

An Inquiry into Preparing Diarrhea Model of Mice and Application of Diarrhea Index

Zhou Gannan, Hu Zhihua, Wang Yaxian, et al

In Order to screen antidiarrhea drugs, the standard operation of mice diarrhea model replicated with leave of Cassia angustifolia vahl was introduced. In experiments, diarrhea index expressed with loose stool incidence rate multiplied by loose stool grade was used as main index. It thoroughly mirrors indi.

vidual diarrhea degree and possesses comparability. The result indicates that diarrhea index in mice presented normal distribution. The index between animal model groups had no difference as well as no difference was found in model mice of same group for six consecutive days, with good reproducibility. It is recommended that the model can be used for the screening of antidiarrhea drugs.

Studies on the Effect of Manyinflorescenced Sweetvetch (*Hedysarum polybotrys*) on Changes of "Qi"–Blood and Acid–Base in RDS Rats

Bai Juan, Qiu Tong, Li Ping, et al

Effect of radix of *Hedysarum polybotrys* on changes of "Qi" (vital energy), blood and acid-base of rats RDS model produced by intravenous injection of oleic acid was measured and observed. The results demonstrated that *H. polybotrys* could markedly increase PaO_2 and O_2Sat, reduce $PaCO_2$, and redress acid-base equilibrium disturbances, which provided a scientific proof of the effect of *H. polybotrys* in the treatment of RDS.

Survey and Protection of Medicinal Resources of Desertliving Cistanche (*Cistanche deserticola*)

Tu Pengfei, He Yanping and Lou Zhicen

The main producing areas of *Cistanche* spp. in Neimongol, Ningxia, Gansu and Xingjiang were surveyed. Plant specimens and samples of 4 species and a new variety, named *C. salsa* var. *albiflora* P. F. Tu et Z. C. Lou, were collected and identified. Their distribution and abundance of resources are reported, and measures of exploiting and protecting comdined with sand-control are suggested.

Physiological and Biochemical Changes During Embryo After–Ripening of American Ginseng (*Panax quinquefolius*) and Effect of Hormones on Such Changes

Li Xianen, Chen Ying and Zhang Jun

In natural condition, two years were needed for *Panax quinquefolius* seeds to break dormancy. In the first year, the embryo developed slowly, and the activities of amylase and peroxidase were low. In the second year the embryo developed rapidly, and the activities of both enzymes also increased gradually. But with the treatment of hormones enzymatic activ·ities accelarated remarkably and speeded up the decomposition of stored nutrients in endos·perm, so that starch and non-reducing sugar decreased while reducing sugar increased rapidly, resulting in earlier germination.

Herbalogical Study on Common Selfheal (*Prunella vulgaris*)

Wang Haibo, Zhang Ziyu, Su Zhongwu, et al

Herbalogical study shows that Xiakucao used in ancient times came from *Prunella vulgaris* and *P. asiatica*. The Portion used for medical purpose in ancient times was its stem and leaf, collected during the period of blooming. The portion used today was the spike, collected approach withering. Modern analytical determination showed that the aerial parts of three *Prunella* species can also be used as medicine when collected in june and july.