大黑蚂蚁及其复方蚁制剂对大鼠睾丸RNA、DNA含量的影响

空军航空医学研究所（北京 100036）　王　忠*　郑学秀　袁国英　张继贵　周晓春　刘保刚　王振国　谷顺才

摘要　80只雄性Wistar大鼠随机分为8组，观察蚂蚁（又名玄驹）提取液及其复方制剂对睾丸RNA、DNA含量的影响。用药21d后将大鼠处死，取睾丸称重，用SDS-苯酚法提取RNA、DNA，以紫外分光光度法定性定量。结果用药组睾丸重量明显高于空白对照（$P<0.01$或0.05）。RNA、DNA含量以空白对照组最低，而各用药组的含量均有不同程度的升高，其中尤以复方蚂蚁液中剂量组、蚂蚁水提取液组及中药组更为明显。

关键词　大黑蚂蚁（玄驹）　复方蚁制剂　睾丸　DNA　RNA

大黑蚂蚁又名玄驹，民间用于强身健体及治疗类风湿性关节炎等疾病[1、2]。笔者在研究中曾发现服用蚁提取物的实验动物睾丸重量增加，曲细精管直径增粗，间质细胞数及周围血清中睾酮水平升高[3]。为了深入研究大黑蚂蚁补肾壮阳的药理作用，我们又进一步分析研究了蚁制剂对大鼠睾丸核糖酸（RNA）及脱氧糖核酸（DNA）的影响。

1　材料与方法

1.1　动物：离乳1个月的Wistar雄性大鼠80只，体重为150～180g，购自军事医学院实验动物中心。

1.2　复方蚂蚁口服液：本所自制。由蚂蚁与中药（淫羊藿、蛇床子、枸杞子等）提取物配制而成。蚂蚁提取物：由蚂蚁提取物制备的应用液。中药提取液：由加入复方蚂蚁口服液中的几位中药提取而成。

1.3　丙酸睾丸素注射液：广州明兴制药厂生产，批号：890923-1，50g/L，使用时用花生油稀释至0.4g/L。

1.4　DNA与RNA测试试剂均由军事医学科学院分子遗传学中心提供。

1.5　方法：将80只大鼠随机分为8组：a）空白对照组。每日给予蒸馏水3.5ml/kg；b）丙酸睾丸素对照组0.2mg/只。c）～e）复方蚂蚁口服液大、中、小剂量组，给药量分别为7.0g/kg、3.5g/kg、1.8g/kg，给药容积为3.5ml/kg；f）蚂蚁水提取液组（简称蚂蚁水液组）：给予与复方蚂蚁口服液所含等量的蚂蚁水提取液1.18g/kg；g）蚂蚁醇提取液组（简称蚂蚁醇液组）：给予与复方蚂蚁口服液所含等量的蚂蚁醇提取液1.18g/kg；h）中药提取液组（简称中药组）：给予与复方蚂蚁口服液所含等量的中药提取液3.32g/kg；除b）组皮下注射给药外，其余各组均为灌胃给药，1次/d，连续21d。第22天将动物处死，迅速摘取双侧睾丸，滤纸吸干血污后，以电子分析天平称重，迅速置液氮中冻存。

1.5.1　RNA，DNA检测方法：由军事医学科学院分子遗传中心协助完成。

1.5.2　RNA的提取：用SDS-苯酚法[4]，在pH5下提取纯化、无水乙醇沉淀后，以备定量测定。

*Address: Wang Zhong, Air Force Institute of Aviation Medicine, Beijing

1.5.3 DNA的提取：用SDS-苯酚法[4]，在pH8.0下提取纯化，并用Rnase处理后，无水乙醇沉淀，以备定量测定。

1.5.4 RNA、DNA纯度及含量测定方法：紫外分光光度检测260nm和280nm的OD值，以其比值定纯度。并按以下公式确定含量。

$$\text{RNA浓度} = \frac{OD260}{0.024 \times L} \times \text{稀释倍数}$$

RNA含量（mg/g）= RNA浓度×溶解V + 乙醇沉淀V × 匀浆V ÷ 组织重量（g）

$$\text{DNA浓度} = \frac{OD260}{0.020 \times L} \times \text{稀释倍数}$$

DNA含量（mg/g）= DNA浓度×溶解V + 乙醇沉淀V × 匀浆V ÷ 重量（g）

2 结果

2.1 用药3周对试验大鼠体重，睾丸重及脏器指数的影响：结果见表1。应用不同剂量的复方蚂蚁口服液与不同方法提取的蚂蚁液，大鼠睾丸重量与脏器指数明显高于空白对照组（$P<0.01$或<0.05）。大剂量组作用与替补雄激素接近。说明该复方蚂蚁口服液与蚂蚁液均有促进幼年鼠睾丸发育的作用。

表1 各用药组大鼠体重、睾丸重及脏器指数比较($\bar{x} \pm S$)

组别	动物数(只)	体重（g）	睾丸重（g）	脏器指数（mg/100g）
空白对照组	10	217.0±24.53	1.67±0.7a	723.95±237.49
复方小剂量组	10	255.6±31.57	2.74±0.44**	1289.26±227.57**
复方中剂量组	10	218.5±48.29	2.32±0.49*	1133.47±247.28**
复方大剂量组	10	229.9±34.08	2.61±0.79*	1131.24±305.17**
蚂蚁水液组	10	215.1±30.30	2.29±0.58*	1035.40±201.20**
蚂蚁醇液组	10	235.5±29.27	2.56±0.45*	939.02±237.59*
中药组	10	229.11±36.63	2.31±0.45*	1020.65±188.01*
丙酸睾丸素组	10	241.9±35.42	2.52±0.57**	1049.30±222.93**

与空白对照组 **$P<0.01$ *$P<.005$

2.2 用药3周对大鼠睾丸RNA含量的影响：结果见表2。空白对照组RNA含量最低(1.51±0.48mg/g)，与其比较各用药组RNA含量均有升高。其中，尤以复方蚂蚁口服液小剂量组与中药组明显，与阳性对照组(丙酸睾丸素组)接近。但由于例数较少，故各组比较统计学差

表2 蚁制剂对大鼠睾丸组织RNA含量的影响($\bar{x} \pm S$)

组别	n	RNA含量(mg/g)	t值	P值*
空白对照组	5	1.510±0.48		
复方小剂量组	5	1.632±0.44	0.419	>0.05
复方中剂量组	5	1.836±0.71	0.851	>0.05
复方大剂量组	5	1.690±0.32	0.698	>0.05
蚂蚁水液组	5	1.632±0.41	0.432	>0.05
蚂蚁醇液组	5	1.762±0.33	0.962	>0.05
中药组	5	2.238±0.80	1.745	>0.05
丙酸睾丸素组	5	2.566±1.34	1.659	>0.05

*与空白对照组比较

表3 蚁制剂对大鼠睾丸组织DNA含量的影响($\bar{x} \pm S$)

组别	n	DNA含量(mg/g)	t值	P值*
空白对照组	5	2.85±0.96		
复方小剂量组	5	2.94±1.39	0.119	
复方中剂量组	5	4.20±2.07	1.323	
复方大剂量组	5	3.94±1.45	1.402	
蚂蚁水液组	5	4.51±1.23	2.379	<0.05
蚂蚁醇液组	5	4.10±1.87	1.329	
中药组	5	4.51±1.20	2.415	<0.05
丙酸睾丸素组	5	4.10±2.11	1.206	

*与空白对照组比较

异不显著。但从中可以看出一用药后升高的趋势。

2.3 用药3周对大鼠睾丸DNA含量的影响：结果见表3。空白对照组DNA含量最低（2.85±0.96mg/g），各组与该组比较，其DNA含量均有所升高，尤以蚂蚁水提液组和中药组比较显著（$P<0.05$），复方蚂蚁口服液小剂量组亦与阳性对照组（丙酸睾丸素）接近。

3 讨论

现在人们越来越重视DNA、RNA的生物学活性。DNA-RNA结构与功能的研究能解释细胞的许多生命现象，而DNA、RNA含量的测定也能在一定程度上说明生命现象。DNA是细胞生物功能及遗传信息的载体，而DNA控制并从DNA模板上转录的RNA具有许多生物学活性。这些物质含量的多少决定着细胞功能的强弱。在睾丸中进入分裂周期的精原细胞越多，则单位重量的DNA含量越多；睾丸间质细胞功能越活跃，则单位重量的RNA含量越多。以往我们用吖啶橙荧光染色的方法已经发现服用蚂蚁提取物的试验动物除能增强免疫功能外，胸腺细胞、淋巴细胞及睾丸间质细胞和精母细胞内的DNA、RNA含量增加。本文进一步检测了应用不同方法提取和不同剂量的大黑蚂蚁提取液与复方蚁制剂的大鼠睾丸中DNA、RNA含量，并作定量分析。再次证明蚁提取液能够使被试动物睾丸重量及其脏器指数增加，睾丸组织中RNA与DNA含量用药后与不用药组比较有一显著增高的趋势。这与我们另一研究成果，即应用蚂蚁制剂4周能够提高性腺重量，分泌睾酮的间质细胞数增多，电镜下见用药组分化旺盛、代谢活跃，血清性激素水平增高的结论相一致。

关于蚂蚁制剂能够增加睾丸组织中的核酸含量的作用机制尚不清楚。初步分析可能与该制剂富含微量元素Mn、Zn、Se及大量人体必需的氨基酸，维生素E和β-胡萝卜素等有关[5]。维生素E缺乏则出现雄鼠睾丸萎缩，不产生精子。雌鼠则会导致胎盘萎缩与流产。Mn可激活RNA与DNA聚合酶，促进核酸的合成[6, 7]。其它元素亦是多种酶类的组分或激活剂，又是维持正常生殖的必需元素。

因此，蚂蚁提取物及其复方制剂对生殖系统与核酸代谢的作用可能是各种因素综合作用的结果。对此有待进一步深入研究。

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(Original article on page 403)

Determination of Matrine in Jieshenbao Lotion by TLC Scanning

Li Xiangyang, Tu Wanqian

TLC scanning technique was developed for the determination of matrine in Jieshenbao lotion. The method is simple, and highly sensitive, with an average recovery of 99.87% and a variation cofficient of 1.33%.

(Original article on page 405)

Antipromoting Tumor and Antioxidant Actions of G_{9315}

Fu Naiwu, Liu Zhaoyang, Zhang Ruyi

G_{9315} was a complex extracted from *Glycyrrhizae inflate* Batal, and consisted of 6 flavonoids with significant antioxidant effects. At 2mg dose, it showed strong antipromoting effect on two stage carcinogenesis in mouse skin induced by DMBA plus croton oil. At 10μg/ml dose, it inhibited the chemiluminescence (CL) of polymorphonuclear leukocyte (PMN) of Wistar rat induced by croton oil, at 20 μg/ml, it inhibited the CL of new born Balb/c mouse epidermis and liver mitochondria induced by croton oil. It also inhibited the CL of Balb/c mouse microsome induced by CCl_4.

(Original article on page 411)

Effects of Dayecai (*Selaginella doedealeinii*) and Chinese Livistona (*Livistona chinensis*) on the activity of Protein Kinase *C*

Huang Cai, Qin Yanmei, Liang Nianci

Protein Kinase C (PKC) was partially purified from rat brain by DEAE-52 column chromatography, and the effects of ethanol extracts of *Selaginella doederleinii and Livistona* chinensis on PKC activity were investigated. In order to verify the reliability of the assay system, the effect of polymyxin B, a known PKC inhibitor, on the activity of PKC was also tested. Results showed that the ethanol extract of *S. doederleinii* strongly inhibited PKC activity with IC50 2.2μg/ml. The ethanol extract of *L. chinensis* (100μg/ml) inhibited PKC activity by 66.6%. Polymyxin B inhibited PKC activity with IC_{50} 17.7 U/ml.

(Original article on page 414)

Effects of Big Black Ant and Its Compound Preparations on the RNA and DNA Levels in Testes of Rats

Wang Zhong, Zheng Xuexiu, Yuan Guoying, et al

80 male Wistar mice were randomly divided into 8 groups. The effects of the big black Ant preparations (alias Xuanju) obtained in different ways and their compound preparations in different dosages on the amount of RNA and DNA in the testes of mice were observed. After administration of the preparations for 21 days, the mice were decapitated and the testes were excised and weighed. The RNA and DNA in the testes were isolated and

purified with the method of SDS- phenol and their amounts were measured with UV-VIS spectrophotometry. The results showed that the mean testes weight in the experimental groups was significantly higher than that in the control group ($P<0.01$ or 0.05).The amount of RNA and DNA in the experimental groups, especially in the medium dose Xuanju compound treated group, Xuanju water extract group and Chinese traditional medicine group were more than that in the control group.

(Original article on page 416)

Pharmacognostical Studies on the Two Ploidy Level of Indigowoad (*Isatis indigotica*)

Qiao Chuanzhuo, Dai Fubao, Cui Xi, et al

Morphological and histological characteristics of the root (Banlangon) and leaf (Daqingye) of *Isatis indigotica* Fort. derived from the autoteraploid (2n = 28) and its parent diploid (2n = 14) were compared macroscopically and microscopically. Results showed that expression of polyploidy giantism resulting from chromosome duplication were evident not only among organs but also among tissues of the same organ. Leaves of the tetraploid contained 1.79~3.11μg/mg more indigo than the diploid near the harvest time.Increase of indirubin was also obvious.polysaccharide in the leave showed no much difference but that in the root was doubled and total amino acid was increased by 4.8%.

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Distinction of Several Pairs of Easily Confusable Herbal Seeds Under Scanning Electron Microscope

Zhang Shuhua, Wang Li, et al

Easily confusable seed of *Ziziphus jujuba* var. *spinosa* (Bunge) Hu and *Hovenia acerba* Lindl.; *Allium tuberosum* Rottl.ex spreng. and *A.fistulosum*;*Sinapis alba* L. and *Brassica campestris* L. were examined under scanning electron microscope. Results showed that; 1.) irregular nests were present on the surface of semen *Z.jujuba* var. *spinosa*, while papillae appeared on the surface of semen *H.acerba* Lindl; 2.) Cells of seed coat of semen *A. tuberosum* were irregularly shaped or polygonic with protruding cutin on its peridium, while that of Semen *A. fistulosum* were polygonic with connecting fibers between cells; 3.) cells of semen *S.alba* L. were similarily polygonic, with thick and protruding vertical perine, while that of semen *B.campestris* L. were also polygonic, but its vertical perine were thiner and less protruding, its peridium shrinked in side and was covered with cutinous nets veins.

(Original article on page 427)

Herbological Studies on "Male" and "Female" Heshouwu

Zhan Xuefeng

The saying that the herbal Heshouwu were present in "male" and "female" forms was studied as for its trivical name, pharmacognosy and drug action.Results of the study showed that the So-called "female" Heshouwu is actually *Cynanchum bungei* Decne and the "male" form is *Polygonum multiflorum*. Coincidently, the historical origin of such saying was briefly discussed.

(Original article on page 431)