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肾虚育精方对肾精亏虚小鼠模型生殖激素及氧化应激水平的影响*

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摘要 目的:研究肾虚育精方对肾精亏虚小鼠模型生殖激素及氧化应激水平的影响。**方法:**选择6周龄雄性健康级SD小鼠60只纳入本次研究。根据随机数字法将小鼠分成3组,分别为观察组、模型组及对照组,每组各20只。观察组及模型组的小鼠通过“房劳和惊恐复合伤肾法”建立肾精亏虚模型,对照组小鼠不建模。建模完成后观察组小鼠每日给予肾虚育精方的浓缩液干预,模型组及对照组则予以等量的生理盐水灌胃。干预21d后对比各组小鼠睾丸组织有关指标,包括Johnsen 10级积分、生精小管的直径及生精上皮厚度,小鼠生殖激素水平,包括血清卵泡刺激素(FSH)及睾酮(TEST),氧化应激指标水平,包括丙二醛(MDA)、过氧化物歧化酶(SOD)及总抗氧化能力(T-AOC)。**结果:**对照组与观察组的Johnsen 10级积分高于模型组,生精小管的直径及生精上皮厚度大于模型组,差异均有统计学意义($P<0.05$);观察组的生精上皮厚度明显大于对照组,差异有统计学意义($P<0.05$)。对照组与观察组小鼠的FSH及TEST水平均明显高于模型组,差异均有统计学意义(均 $P<0.05$)。对照组与观察组的MDA和SOD水平明显低于模型组,T-AOC水平明显高于模型组,差异均有统计学意义($P<0.05$)。**结论:**肾虚育精方能够提升肾精亏虚小鼠模型的生殖激素水平,并降低氧化应激水平,可将肾虚育精方应用于临床治疗。

关键词:肾虚育精方;肾精亏虚;小鼠;生殖激素;氧化应激

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Effects of Shenxu Yujing Recipe on Reproductive Hormone and Oxidative Stress in Mice Model of Kidney Deficiency*

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ABSTRACT Objective: To study the effects of Shenxu Yujing recipe on reproductive hormone and oxidative stress in mice model of kidney deficiency. **Methods:** 60 male healthy SD mice with 6 weeks were included in the study. According to the random number method, the mice were divided into 3 groups, which were the observation group, the model group and the control group, with 20 mice in each group. The mice in the observation group and the model group were established with the kidney deficiency model by the combination of sexual strain and terrified, the mice in the control group were not modeled. After the modeling, the mice in the observation group were given the concentrated solution of the Shenxu Yujing recipe every day, the model group and the control group were given the same amount of normal saline. After the intervention of 21d, the relative indexes of testis tissue in each group were compared, which were included Johnsen 10 stage integral, the diameter of the seminiferous tubules, thickness of seminiferous epithelium, and reproductive hormone levels in mice, including the serum follicle stimulating hormone (FSH) and testosterone (TEST), oxidative stress index, including malondialdehyde (MDA), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC). **Results:** The Johnsen 10 stage integral in the control group and the observation group was higher than that in the model group, the diameter of seminiferous tubules and the thickness of seminiferous epithelium were higher than those of the model group, the differences were statistically significant ($P<0.05$). The levels of FSH and TEST in the control group and the observation group were significantly higher than those in the model group, the differences were statistically significant ($P<0.05$). The levels of MDA and SOD in the control group and the observation group were significantly lower than those in the model group, and the level of T-AOC was significantly higher than that in the model group, the differences were statistically significant ($P<0.05$). **Conclusion:** The Shenxu Yujing recipe can raise the level of reproductive hormone in mice model of kidney essence deficiency, and reduce the oxidative stress level, which can be used in clinical treatment.

Key words: Shenxu Yujing recipe; Kidney deficiency; Mice; Reproductive hormone; Oxidative stress

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前言

肾精亏虚又可称为肾精不足,是指生殖发育等方面功能下降所导致的病症。近年来,临床肾精亏虚病例越来越多,此类患者生殖激素以及氧化应激等水平均受到一定影响,严重干扰其身体健康以及日常生活。临床研究表明,肾精亏虚发病原因包括先天性发育不良、后天性房事过度以及大病伤及肾脏等^[1]。同时相关调查显示,随着社会的进步以及经济的发展,使得现代男性承受了较大的社会和生活压力,这些压力在一定程度上会引起男性生殖能力下降^[2]。因此,寻找出有效的治疗方法,提高肾精亏虚的临床治疗效果是当前研究工作的重点。中医对于肾精亏虚有着十分悠久的历史,认为该病主要致病原因为真阴不足以及精髓内亏,因此,治疗的关键在于补肾填精。有报道指出,肾虚育精方能够发挥补气补血以及温肾壮阳等作用,且有助于改善患者生殖激素以及氧化应激水平,适于推广应用^[3]。本文通过研究分析肾虚育精方对肾精亏虚小鼠模型生殖激素及氧化应激水平的影响,旨在为临床治疗提供相应的数据支持,现报道如下。

1 材料与方法

1.1 实验动物

选择6周龄雄性健康级SD小鼠(购于重庆医科大学附属口腔医院)60只纳入本次研究,许可证号:SYXK(渝)2013-0002。体重为30~35g。根据随机数字法将小鼠分成3组,分别为观察组、模型组及对照组各20只。其中观察组的体重为30~34g,平均 (31.88 ± 1.24) g。模型组的体重为31~35g,平均 (31.79 ± 1.32) g。对照组的体重为30~35g,平均 (32.01 ± 1.40) g。各组小鼠一般资料比较差异无统计学意义($P>0.05$)。医院的动物实验伦理委员会已经批准了此次研究。

1.2 研究方法

1.2.1 建立肾精亏虚小鼠模型 观察组及模型组的小鼠通过“房劳和惊恐复合伤肾法”建立肾精亏虚模型,具体步骤为:两组小鼠每日按1:2的比例同动情期的雌鼠(来源同雄性小鼠)合笼,于每日夜晚20:00往笼内投入雌鼠,次日晨间8:00将雌鼠取出,再于次日夜晚20:00投入雌鼠,至次日晨间8:00将雌鼠取出,如此反复,使雄性小鼠房劳伤肾。另于每日8:30~19:30,将雄性小鼠投入特制铁丝笼内,并将此笼固定于内含1只成熟家猫的大笼中,使猫与小鼠只隔有1层铁丝网,并可使猫爪触碰到却无法抓住小鼠。且在每日的上午10:00及下午15:00另取额外2只活鼠喂猫示众,促使笼中雄性小鼠惊恐伤肾。利用此法连续进行21d,建立好肾精亏虚小鼠模型。对照组小鼠不建模,三组小鼠均常规自由饮食。

1.2.2 药物处理 建模完成后观察组小鼠每日给予肾虚育精方的浓缩液干预,剂量为0.16mL/10g体重。其中肾虚育精方为:熟地黄12g,制首乌12g,枸杞子10g,淫羊藿10g,由医院实验室煎煮至浓缩液,达到含生药量0.8g/mL的标准。模型组及对照组则予以等量的生理盐水灌胃。从造模完成后1d开始干预,共处理21d。

1.2.3 指标检测 干预21d后,将各组小鼠均行颈椎脱臼处死,断尾法提取血液,放进聚合酶链式反应(Polymerase Chain

Reaction, PCR)管内,实施10min 4000 r/min的离心后提取血清。而后打开小鼠腹腔,将右侧睾丸取出,利用Bouin液对其固定24h后石蜡包埋,行HE染色。再将左侧睾丸取出,应用4℃下预冷的锇酸电镜液固定24h,再行超薄切片。睾丸组织的病理学评分通过Johnsen 10级积分法实施评定,于400倍光镜下为每张切片任选20个生精小管实施评分。共分10级,所得积分越高,则表示精子形成越好。再通过购自武汉清平影像技术公司的HMIAS-2000型彩色医学图文系统检测各组小鼠生精小管的直径及生精上皮厚度,每张切片在400倍光镜下任选20个视野,计数后取其平均值记为该切片的生精小管的直径及生精上皮厚度。取部分睾丸组织,称重并通过玻璃匀浆器进行匀浆,为每克组织添加10mL的生理盐水制成10%的匀浆液,再实施10min 4000 r/min的离心,而后提取上清液放于4℃下保存待测。丙二醛(Malondialdehyde, MDA)水平通过硫代巴比妥酸法进行检测,过氧化物歧化酶(Superoxide dismutase, SOD)活性通过黄嘌呤氧化酶法进行检测,总抗氧化能力(Total antioxidant capacity, T-AOC)通过化学比色法进行检测,有关试剂盒均购于南京建成生物研究所。将断尾法提取的血清应用放射免疫法测定血清卵泡刺激素(Follicle-stimulating hormone, FSH)和睾酮(Testosterone, TEST)水平,有关试剂盒均购于北方生物技术研究所,操作步骤严格遵守试剂盒的说明书进行。

1.3 观察指标

对比各组小鼠睾丸组织有关指标(Johnsen 10级积分、生精小管的直径及生精上皮厚度)、小鼠生殖激素水平(FSH、TEST)、氧化应激指标水平(MDA、SOD及T-AOC)。

1.4 统计学方法

通过SPSS21.0统计软件实施数据的处理分析,计数资料用(n,%)表示,其比较选用 χ^2 检验。计量资料用 $(\bar{x} \pm s)$ 表示,其比较选用t检验。多组间数据资料的比较采用方差分析,计算F值, $P<0.05$ 为差异有统计学意义。

2 结果

2.1 各组小鼠睾丸组织有关指标的对比

各组小鼠的Johnsen 10级积分、生精小管的直径及生精上皮厚度整体比较差异均有统计学意义(均 $P<0.05$)。对照组与观察组的Johnsen 10级积分高于模型组,生精小管的直径及生精上皮厚度大于模型组,差异均有统计学意义($P<0.05$)。观察组的生精上皮厚度明显大于对照组,差异有统计学意义($P<0.05$),但两组的Johnsen 10级积分和生精小管的直径比较差异无统计学意义($P>0.05$),见表1。

2.2 各组小鼠生殖激素水平的对比

各组小鼠的FSH、TEST水平整体比较差异均有统计学意义($P<0.05$)。对照组与观察组小鼠的FSH、TEST水平均明显高于模型组,差异均有统计学意义($P<0.05$)。观察组与对照组的FSH、EST水平比较差异均无统计学意义($P>0.05$),见表2。

2.3 各组小鼠氧化应激指标水平的对比

各组小鼠的MDA、SOD及T-AOC水平整体比较差异均有统计学意义(均 $P<0.05$)。对照组与观察组的MDA和SOD水平明显低于模型组,T-AOC水平明显高于模型组,差异均有统计学意义($P<0.05$)。观察组与对照组的MDA、SOD及

T-AOC 水平相比差异均无统计学意义(均 $P>0.05$),见表 3。

表 1 各组小鼠睾丸组织有关指标的对比($\bar{x}\pm s$)

Table 1 Comparison of related indexes of testis tissue in mice of each group($\bar{x}\pm s$)

Groups	n	Johnsen 10 stage integral (score)	Diameter of seminiferous tubules(μm)	Thickness of seminiferous epithelium(μm)
Observation group	20	9.48 $\pm$ 0.42*	125.96 $\pm$ 16.88*	29.57 $\pm$ 4.82* ^a
Model group	20	8.95 $\pm$ 0.57 ^a	115.31 $\pm$ 14.25 ^a	26.18 $\pm$ 5.49 ^a
Control group	20	9.62 $\pm$ 0.33	127.62 $\pm$ 18.84	33.72 $\pm$ 6.54
F	-	3.954	12.631	5.872
P	-	0.023	0.000	0.000

Note: compared with model group, * $P<0.05$; compared with control group, ^a $P<0.05$.

表 2 各组小鼠生殖激素水平的对比($\bar{x}\pm s$)

Table 2 Comparison of reproductive hormone levels in mice of each group($\bar{x}\pm s$)

Groups	n	FSH(U/L)	TEST(ng/mL)
Observation group	20	0.09 $\pm$ 0.03*	4.14 $\pm$ 1.50*
Model group	20	0.05 $\pm$ 0.03 ^a	0.62 $\pm$ 0.31 ^a
Control group	20	0.10 $\pm$ 0.02	4.71 $\pm$ 2.84
F	-	3.588	5.121
P	-	0.039	0.000

Note: compared with model group, * $P<0.05$; compared with control group, ^a $P<0.05$.

表 3 各组小鼠氧化应激指标水平的对比($\bar{x}\pm s$)

Table 3 Comparison of indexes of oxidative stress in mice of each group($\bar{x}\pm s$)

Groups	n	MDA($\mu\text{mol/gprot}$)	SOD(U/m gprot)	T-AOC(U/m gprot)
Observation group	20	15.79 $\pm$ 1.52*	115.03 $\pm$ 11.28*	4.27 $\pm$ 1.43*
Model group	20	24.27 $\pm$ 6.01 ^a	122.94 $\pm$ 10.65 ^a	2.73 $\pm$ 0.48 ^a
Control group	20	16.55 $\pm$ 1.43	114.78 $\pm$ 12.89	4.34 $\pm$ 1.46
F	-	6.821	11.237	4.931
P	-	0.000	0.000	0.001

Note: compared with model group, * $P<0.05$; compared with control group, ^a $P<0.05$.

3 讨论

男性肾精亏虚主要临床表现包括眩晕耳鸣,并伴有腰膝酸软,精子量少以及性功能减退等。中医认为,肾所藏之精乃生命活动之根本。同时,肾精具有主生长繁殖的重要功能,因此可作为人体的一项基础物质^[4,5]。此外,肾精不但可以调节脏腑,满足活动所需,还可以参与机体生髓、养骨以及生血等活动,从而提升机体抵抗力。同时,肾精对于男性而言十分重要,如男性出现肾精不足,则其自身生殖能力会受到严重影响,表现为生殖激素水平下降,严重者甚至将对其子代产生不良影响^[6,7]。临床对于肾精亏虚男性患者通常给予补肾育精治疗,进而确保其肾精充足,既有助于提高其生殖能力,同时也有利于降低子嗣夭弱风险。多项报道表明,精子的生成是一个十分复杂的过程,FSH 和 TEST 等生殖激素是启动及维持精子生成的关键性激素;同时肾精亏虚男性患者抗氧化能力会显著下降,进而导致其氧化应激能力明显上升,而补肾育精相关治疗能否对生殖激素以及

氧化应激等水平进行有效改善,目前尚无确切定论^[8-10]。

本文研究发现,对照组与观察组的 Johnsen 10 级积分高于模型组,生精小管的直径及生精上皮厚度大于模型组,差异均有统计学意义($P<0.05$),观察组的生精上皮厚度明显大于对照组,差异有统计学意义($P<0.05$),这提示了应用肾虚育精方能有效改善肾精亏虚小鼠睾丸组织的形态,对精子的形成具有较好的促进作用。分析原因可能与肾虚育精方的药理作用机制有关。肾虚育精方主要中药组成为熟地黄、制首乌以及枸杞和淫羊藿等,其中熟地黄是君药,具有补肾填精之功效,而制首乌能够益精补血,枸杞子有助于育精固髓,此二者和熟地黄合用,可提高育精疗效。淫羊藿利于育精气,固筋骨,可帮助补肾壮阳。本方将补阳以及育精药物合用,起到益肾生精的疗效。同时,本研究发现,对照组与观察组小鼠的 FSH、TEST 水平均明显高于模型组($P<0.05$),但观察组与对照组的 FSH、TEST 水平比较差异均无统计学意义($P>0.05$)。这提示了肾虚育精方还可明显提升小鼠的生殖激素水平。原因主要可能与肾虚育精方的补肾养

精作用有关。中医认为恐为肾志,即过于惊恐则会伤及肾脏。现代医学研究发现情绪应激会对人体神经系统以及内分泌系统造成严重影响,从而导致调节紊乱,进而影响生殖能力^[11-13]。FSH 为有利于支持细胞分化、增殖等活动的启动因子,其不但可以提升性腺功能,促使机体提高生精能力,同时有利于精子数量、质量维持在正常水平^[14-16]。肾精亏虚小鼠模型 FSH 活性受到阻断,因此表现为支持细胞数量下降,且结构异常,进而引发生精细胞减少,且血清 TEST 以及雄激素等均显著下降,导致生育能力降低^[17]。TEST 可直接影响男性精子发生,当缺少 TEST 时,男性则会表现为少精甚至无精等情况^[18]。本研究中医肾精亏虚小鼠模型表现为 TEST 含量显著下降。当给予肾虚育精方治疗之后,FSH 及 TEST 水平明显改善,这也说明了肾虚育精方具有较好的补肾养精作用。此外,本研究还发现,对照组与观察组的 MDA 和 SOD 水平明显低于模型组,T-AOC 水平明显高于模型组 ($P<0.05$),但观察组与对照组的 MDA、SOD 及 T-AOC 水平比较差异均无统计学意义 ($P>0.05$)。这提示了肾虚育精方对肾精亏虚小鼠的氧化应激水平也具有较好的改善作用。分析原因可能是因为与肾虚育精方具有的拮抗氧化应激作用有关。具体而言,临床已经证实人类精子能够产生活性氧片段,并且此片段和精子运动能力等自身生理状况息息相关,同时生殖道的活性氧片段会对睾丸功能产生一定影响,进而降低其精子质量。因此,活性氧作为负性影响,不但会对睾丸以及精子造成氧化损伤,同时还会触发多种细胞损伤机制。研究证实,活性氧片段会导致患者睾丸间质过氧化,使人体分泌能力降低,从而导致 TEST 水平下降,同时其也会造成支持细胞受损,进而引发患者不育的严重后果^[19]。SOD 为需氧生物以及耐氧生物共同拥有的氧化酶,可用于清除氧自由基^[20]。因此,SOD 对于活性氧片段的清除过程十分重要,可作为细胞中自由基的关键排污槽。此外,机体对于抗氧化系统主要分为酶促以及非酶促两种,而非酶促反应依靠维生素和氨基酸等完成。以上两类防御体系整体被称作 T-AOC。而 MDA 则为过氧化脂质的一种分解产物,并用于判断氧化应激程度。本研究中肾精亏虚小鼠模型 MDA 水平上升,证实该类小鼠睾丸中脂质过氧化反应加剧,超氧化反应十分活跃。通过应用肾虚育精方补肾之后,其肾虚导致的氧化损伤得到显著拮抗,说明肾虚育精方具有较好的治疗效果。

综上所述,肾虚育精方对肾精亏虚小鼠模型生殖激素及氧化应激水平均具有较好的改善作用,可考虑将肾虚育精方用于临床治疗,从而更好地为患者服务。

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