PATHOLOGICAL STUDY ON THE CHRONIC TOXICITY OF THAI TRADITIONAL MEDICINE: YA-RID-SI-DUANG-MAHAKAL

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Abstract

The pathological study on chronic toxicity of Thai traditional medicine: Ya-rid-si-duang-mahakal was investigated in 180 wistar rats. The rats were divided into 6 groups, 30 rats in each group (male = 15 and female = 15). Group 1 was a control group, treated orally with distilled water. Group 2-5 were treated orally with 10, 100, 500 and 1000 mg/day/kg body weight for 6 months continuously. Group 6 (Recovery group), they were treated orally with 1000 mg/day/kg body weight for 6 months as treatment group. After that, they were then stopped administration 2 weeks before euthanasia. The histopathological results of the treatment groups showed significant lesions in kidneys and liver when compared to control ($p<0.05$). The kidneys showed various tubular casts with mild to moderate degree tubulonephrosis and moderate degree focal nephritis as well. Liver showed mild to moderate degree fatty degeneration with moderate degree focal hepatic necrosis. There were some reconizable lesions in other organs, such as, submucosal edema of stomach, laryngeal epithelial hyperplasia when the rats were given in high-dose groups. In conclusion, the chronic toxicity in 6 months administration of Ya-rid-si-duang-mahakal did not demonstrate any pathological changes in the visceral organs of the experimental rats. However, the kidney and liver function test should be monitored in the long term administration.

Keywords: pathological study, chronic toxicity, Thai traditional medicine, Ya-rid-si-duang-mahakal

Introduction

Hemorrhoid is the common health problem in human. The Thai traditional medicine: Ya-rid-si-duang-mahakal is one of alternative medicine that practically use in many hospitals for treatment1. The study of mutagenicity of Ya-rid-si-duang-mahakal has been previously described1. There has been neither toxicity test nor adverse effect of this Thai traditional medicine reported yet. Therefore, this study is attempted for study on pathology on chronic toxicity

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of Ya-rid-si-duang-mahakal in experimental rats. The obtained data are useful for the patient on safety and risk assessment of Ya-rid-si-duang-mahakal as a medical treatment for hemorrhoid in the primary health care.

Materials and Methods

Preparation of the Medicine

The Thai traditional medicine:Ya-rid-si-duang-mahakal was in powdered preparation without mercuric sulfide composition. The crude ingredients were extracted by 95% alcohol 600 ml/ kg, then filtered and evaporated by rotary evaporator. The extraction residues were added by distilled water 600 ml, filtered and evaporated by lyophilizer. The preparations were then mixed, dissolved with distilled water and adjusted in various concentrations for chronic toxicity test1.

Animals and Chronic Toxicity Study

One hundred eighty wistar rats weighing 200 ± 10 g. for male and 180 ± 10 g. for female were purchased from National Laboratory Animal Center, Mahidol University. They were housed for acclimatization in experimental room at 25±1°C, 60% humidity and 12 hours/day artificial light. The mice were fed with sterile food and water *ad lib*.

Procedures for chronic toxicity were as followed. The rats were divided into 6 groups, 30 rats in each group (male = 15 and female = 15). Group 1 was a control group, treated orally with distilled water as placebo effect. Group 2-5 were treated orally with 10, 100, 500 and 1000 mg/day/kg body weight for 6 months continuously. For group 6 (Recovery group), the rats were treated orally with 1000 mg/day/kg body weight for 6 months as treatment group. They were stopped the medicine administration for 2 weeks before necropsy. At the end of the experimental period, the rats were euthanized by ether inhalation. During experimental period, the necropsy was then performed in the dead rats.

Upon necropsy the gross and histopathological lesions were determined[2]. The organs (brain, heart, lung, liver, kidney, larynx, spleen, intestine, pancreas, testis, prostate gland, seminal vesicle, ovary, uterine, mammary gland, salivary gland, lacrimal gland, thyroid gland, adrenal gland and bone marrow) were macroscopically examined. The tissues were then preserved in 10% buffer formalin and routinely processed for histology technic[3]. The tissues were embedded in paraffin block and cut into 5 microns thickness. The slides were stained by Hematoxylin & eosin (H&E stain) and observed under light microscope. In some section, the special stains such as Alcian blue pH 3, Prussian blue and Von Kossa were also employed.

Statistic analysis

The degrees of pathological severity were determined in all organs. There were mild, moderate and severe. No remarkable lesion (NRL) was used in case of no recognizable pathological change. The pathological lesion data in male and female (non-parametric) were compared between control and experimental groups. The statistical analysis used Mann Whitney Test ($p < 0.05$) by program (SPSS version 14.0)

Results

No remarkable gross lesion was observed in the internal organs. The histopathological results on chronic toxicity of Thai traditional medicine: Ya-rid-si-duang-mahakal were shown (Table 1 and 2). There were mild and moderate degree, and no remarkable lesion (NRL) in liver, kidney, spleen, adrenal gland, brain, bone marrow, larynx, pancreas, stomach, intestine, heart and lung. There were NRL in other organs, such as testis, prostate gland, seminal vesicle,

ovary, uterine, mammary gland, salivary gland, lacrimal gland, and thyroid gland. Moreover, there were pulmonary congestion, focal pulmonary emphysema and mild degree bronchiolar associated lymphoid tissue (BALT) hyperplasia in all groups which showed no statistical significant ($p>0.05$). The pathological lesions that showed statistical significant in each group ($P<0.05$), were in kidneys and liver. There were mild to moderate degree tubulonephrosis with renal cast accumulation. The casts were protein, hyaline and calcium cast in renal tubules. Mild to moderate degree of hepatic cell degeneration and focal hepatic necrosis found in treatment groups. The others were splenic hemosiderosis, laryngeal epithelial hyperplasia, gastric submucosal edema and adrenal cortex hyperplasia (Fig. 1 a,b,c,d,e,f.). The necropsy results of the three rats that died during the experimental period; two female rats in control and 500 mg/day/kg body weight and one male rat in 1000 mg/day/kg body weight were respiratory and circulatory failure.

Discussion

The pathological lesions that showed statistical significant in treatment groups when compared to control group, mainly demonstrated in kidney and liver. In addition, the lesions in stomach, larynx were well pronounced when rat received high dose of the medicine that could be due to chronic irritation[4]. For 6 months administration in various doses effected on the renal tubular epithelium. There were renal tubular degeneration with various degrees of cast accumulation which were due to nephrotoxic effect[5]. The degree was also consistently increased when the rats were treated with high dose. Mild to moderate degree hepatic cell degeneration and focal hepatic necrosis were found in all group and high dose group that could be the hepatotoxic effect[6-7]. It has been reported that hepatic cells in rat show rapidly regeneration that could also compensate the mild degree pathological changes[8-9]. It was revealed that three rats died during the experimental period caused by circulatory failure or shock[4,10-11]. The splenic hemosiderosis also found in all groups and showed more severity in high-dose group. It was resulted from the erythrocyte destruction or lysis of erythrocyte that occurred in normal physiology or mild toxic condition[4-6]. The adrenal cortex hyperplasia renders the hyperadrenocoticism that cause by the continuous stress[4]. Upon the results, the 6 months admistration of Ya-rid-si-duang-mahakal did not demonstrate any pathological changes in visceral organs of both male and female rats in all groups. However, it is suggested that the kidney and liver function test should be monitored in the long term administration.

Table 1 The Histopathological findings in male rats (n=90)

	Doses (mg/day/kg body weight)					
	0	**10**	**100**	**500**	**1000**	**1000-R***
	(n=15)	**(n=15)**	**(n=15)**	**(n=15)**	**(n=15)**	**(n=15)**
Mild degree tubulonephrosis	+ 20%	+ 66.7%	+ 13.3%	+ 53.3%	+ 100%	+ 73.3%
Focal nephritis				+# 40%	+# 13.3%	+# 40%
Moderate degree tubulonephrosis with casts	+ 26.7%	+ 53.3%	+ 60%	+# 100%	+# 100%	+# 100%
Mild degree focal hepatic necrosis				+# 73.3%	+# 46.7%	+# 60%
Moderate degree hepatic fatty degeneration				+# 86.7%	+# 80%	+# 86.7%
Mild degree splenic hemosiderosis	+ 100%	+ 100%	+ 100%	+ 100%	+ 100%	+ 100%
Gastric submucosal edema	+ 40%	+ 46.7%	+# 26.7%	+# 60%	+# 33.3%	+# 33.3%
Laryngeal epithelial hyperplasia	+ 40%	+# 86.7%	+# 86.7%	+# 93.3%	+# 93.3%	+# 93.3%

*1000-R = recovery group, + pathological lesions,

#statistical significant when compared to control group ($p<0.05$)

Table 2 The Histopathological findings in female rats (n=90)

	Doses (mg/day/kg body weight)					
	0 (n=15)	10 (n=15)	100 (n=15)	500 (n=15)	1000 (n=15)	1000-R* (n=15)
Mild tubulonephrosis	+ 80%	+ 53.3%	+ 73.3%	+ 40%	+ 43.3%	+ 73.3%
Focal nephritis				+# 86.7%	+# 80%	+# 86.7%
Mild degree tubulonephrosis with casts	+ 46.7%	+ 53.3%	+ 53.3%	+# 100%	+# 100%	+# 100%
Periportal cellular infiltration				+# 86.7%	+# 60%	+# 80%
Mild splenic hemosiderosis	+ 100%	+ 100%	+ 100%	+ 100%	+ 100%	+ 100%
Gastric submucosal edema	+ 26.7%	+# 40%	+# 40%	+# 46.7%	+# 26.7%	+# 100%
Laryngeal epithelial hyperplasia	+ 33.3%	+# 73.3%	+# 86.7%	+# 53.3%	+# 86.7%	+# 86.7%

*1000-R = recovery group, + pathological lesions,

#statistical significant when compared to control group ($p<0.05$)

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การศึกษาทางพยาธิวิทยาความเป็นพิษเรื้อรังของตำรับยาไทย: ยาริดสีดวงมหากาฬ

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บทคัดย่อ

ศึกษาความเป็นพิษเรื้อรังของตำรับยาไทยยาริดสีดวงมหากาฬในหนูถีบจักร พันธุ์วิสตาร์จำนวน 180 ตัว โดยแบ่งหนูออกเป็น 6 กลุ่ม กลุ่มละ 30 ตัว ประกอบด้วยเพศผู้ 15 ตัวและเพศเมีย 15 ตัว กลุ่มที่ 1 เป็นกลุ่มควบคุมได้รับน้ำกลั่นทางปาก กลุ่ม 2 - 5 ได้รับยาทางปากในขนาด10 100 500 และ 1000 มิลลิกรัม/วัน/น้ำหนักตัว 1 กิโลกรัม เป็นระยะเวลาติดต่อกัน 6 เดือน กลุ่มที่ 6 ได้รับสารสกัดทางปากในขนาด 1000 มิลลิกรัม/วัน/น้ำหนักตัว 1 กิโลกรัม ติดต่อกัน 6 เดือน และหยุดยา 2 สัปดาห์ก่อนทำการชันสูตรซาก ผลการศึกษาทางพยาธิวิทยาพบรอยโรคที่ตับและไตอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) เมื่อเปรียบเทียบในกลุ่มควบคุมและกลุ่มได้รับยา ไตพบตะกอนสะสมในท่อไต ร่วมกับภาวะท่อไตเสื่อมสภาพในระดับอ่อนถึงระดับปานกลาง และการอักเสบแบบหย่อมในไตระดับปานกลาง พบเซลล์ตับตายแบบหย่อมและการเสื่อมสภาพของเซลล์ตับแบบมีไขมันแทรกในระดับอ่อนถึงระดับปานกลาง พบรอยโรคในอวัยวะอื่นๆได้แก่การบวมน้ำใต้เยื่อบุกระเพาะอาหาร และเซลล์เยื่อบุกล่องเสียงบริเวณคอหอยเพิ่มจำนวนและขยายใหญ่ในระดับอ่อนถึงปานกลาง เมื่อหนูทดลองได้รับยาขนาดสูงขึ้นด้วย จากผลการศึกษาสรุปได้ว่าการได้รับยาริดสีดวงมหากาฬในขนาดต่างๆติดต่อกันเป็นระยะเวลา 6 เดือนไม่พบพยาธิสภาพในอวัยวะภายในหนูทั้งสองเพศ แต่ควรมีการเฝ้าระวังโดยการตรวจการทำงานของตับและไตอย่างต่อเนื่องหากได้รับยาเป็นเวลานาน

คำสำคัญ: การศึกษาทางพยาธิวิทยา พิษเรื้อรัง ตำรับยาไทย ยาริดสีดวงมหากาฬ

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