

Acute to Subchronic Toxicity and Reproductive Effects of Aqueous Ethanolic Extract of Rhizomes of *Lasia spinosa* Thw. in Male Rats

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Abstract

Acute and subchronic tests on the toxicity and reproductive effects of aqueous ethanolic extract of rhizomes of *Lasia spinosa* Thw. was studied in male rats. In the acute test, oral administration of 5, 10, 20, 40 g/kg of the extract showed no mortality or behavioral changes. In the subchronic test, administration of 5 or 20 g/kg of the extract for 28 days revealed no mortality of the animals. No differences in hematological parameters were observed in either control or treatment groups of both tests. For blood chemistry analysis in both tests, triglycerides in treatment rats were significantly decreased compared to the control ones. However, no significant changes occurred in other parameters. There was no significant difference in the weight of whole body and organ between the control and treatment animals of both acute and subchronic tests. Pathologically, no significant changes of gross and histopathology were observed in both the control and treatment rats. For reproductive effects, significant increase in testicular weight and epididymal sperm count was observed. However, no changes in serum testosterone were found. In summary, oral administration of the extract of *L. spinosa* produced no toxic changes in acute and subchronic toxicity tests. Moreover, the extract had potential for increasing reproductive function in male rats.

Keywords: *Lasia spinosa*, rat, reproductive effects, rhizome, toxicity

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บทคัดย่อ

การศึกษาความเป็นพิษและผลกระทบต่อระบบสืบพันธุ์ของหนูแรทเพศผู้จากการได้รับสารสกัดจากراكผักหนามในระยะเฉียบพลันและกึ่งเรื้อรัง

ธีระยุทธ แก้วออมวงศ์¹ วรรณาภิภา สุทธิไกร³ อนงค์ บินทวิหค² วิจิตร บรรลุนารา^{1*}

การศึกษาความเป็นพิษและผลกระทบต่อระบบสืบพันธุ์ในระยะเฉียบพลันและกึ่งเรื้อรังของหนูแรทเพศผู้จากการได้รับสารสกัดจากراكผักหนาม ในการทดลองระยะเฉียบพลัน ได้ทำการป้อนสารสกัดขนาด 5 10 20 40 กรัมต่อน้ำหนักตัว 1 กิโลกรัม พบร้าไม่มีการตายและการเปลี่ยนแปลงทางพฤติกรรมใดๆ ในสัตว์ทดลอง ส่วนการทดลองของผลกระทบในระยะกึ่งเรื้อรัง ได้ทำการป้อนสารสกัดขนาด 5 และ 20 กรัมต่อน้ำหนักตัว 1 กิโลกรัม เป็นเวลา 28 วัน พบร้าไม่มีการเปลี่ยนแปลงของค่าโลหิตวิทยา สำหรับค่าทางเคมีของเลือดพบการลดลงของไตรกลีเซอไรด์ในกลุ่มที่ป้อนสารสกัดจากรากผักหนาม ส่วนการศึกษาทางพยาธิวิทยา ไม่พบรอยโรคที่แตกต่างของทั้งกลุ่มควบคุมและกลุ่มป้อนสารสกัด ผลการทดลองต่อระบบสืบพันธุ์พบว่า มีการเพิ่มขึ้นของน้ำหนักของอวัยวะและจำนวนอสุจิในกลุ่มที่ป้อนสารสกัดอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับกลุ่มควบคุม แต่ไม่พบการเพิ่มขึ้นของระดับฮอร์โมนโปรเจสเตรอร์โโนอย่างมีนัยสำคัญ ดังนั้นการให้สารสกัดจากراكผักหนามโดยการป้อนในหนูแรทในระยะเฉียบพลันและกึ่งเรื้อรังไม่ก่อให้เกิดความเป็นพิษ และมีผลในการส่งเสริมการทำงานของระบบสืบพันธุ์เพศผู้เพิ่มขึ้น

คำสำคัญ: ผักหนาม หนูแรท ผลต่อระบบสืบพันธุ์ راك ความเป็นพิษ

¹ภาควิชาพยาธิวิทยา ²ภาควิชาเภสัชวิทยา ³โครงการศูนย์นวัตกรรมเทคโนโลยีชีวภาพทางสัตวแพทย์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330

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Introduction

Lasia spinosa Thw. (Araceae) is a perennial herb in moist and shaded areas along rivers in evergreen forest widespread throughout the region of Thailand, where it is commonly known as "Phak Naam" in Thai. Both rhizomes and leaves of *L. spinosa* are commonly used in folk medicine for antitussive, expectorant and relief of abdominal pain. Moreover, the stem is used to relieve itch from roseolar infantum, measles, rubella and other skin diseases (Boonyaprapat and Chokchaijarunporn, 1996). In veterinary field, *L. spinosa* is used locally for increasing the libido of male animals. In large ruminants, *L. spinosa* is also fed to improve growth rate and feed efficiency. Suthikrai and colleagues (1996 and 2006) reported previously that testosterone (T) was a major chemical constituent of *L. spinosa*. Recently, our research group found that using the concentrate dry powder of *L. spinosa* (30 g/head/day) as feed additive could increase growth rate and decrease plasma oestradiol 17- β and progesterone in swamp buffaloes and Murrah x Swamp buffalo cross-bred calves (Suthikrai et al., 2007). Despite the wide use of *L. spinosa* in Thai folk medicine and veterinary medicine, no toxicity study has been reported. Therefore, the purpose of the present study was to

evaluate acute and subchronic oral toxicity of the aqueous ethanolic extract from rhizomes of *L. spinosa* in male Sprague-Dawley rats. In addition, effects on male reproductive function and serum testosterone level of animals were also determined.

Materials and Methods

Plant material: Herbarium specimens from Department of Botany, Faculty of Science, Chulalongkorn University, Thailand were used to compare with our rhizomes of *L. spinosa* for authentication. Mixture of the fresh rhizomes of *L. spinosa* were dried at 40°C in hot air oven with 10-13% moisture and ground to powder.

Preparation of the extract: The obtained powder (100 g) was blended in 100 ml of 4:1 ethanol/water mixture for 48 hr, followed by filtration and concentration with vacuum evaporator. The freeze-dried extract powder was stored at -20°C until use. The major chemical constituents were determined according to the RIA procedure (Kamonpatana et al., 1979; Kamonpatana, 1986). The testosterone level was 8.42±1.2 pg/g of powder.

Experimental animals: All laboratory animals were purchased from National Laboratory Animal Centre, Mahidol University, Thailand. Thirty-eight male

Sprague-Dawley rats (10 weeks old, 250-280 g body weight) were used in acute, subchronic oral toxicity tests and reproductive effect evaluation.

Animal facility under the condition of 12:12 hour light-dark cycle, 24±1°C, 55±10% relative humidity and negative atmospheric pressure was used to house the animals. All animals were supplied with standard pellets and tap water *ad libitum* during the testing period. All experiments were proved by the ethics committee of Chulalongkorn University Animal Care and Use Committee (CU-ACUC; Approval No. 1031005).

Short term reproductive efficacy test: Twenty male rats were randomly assigned into four groups (n = 5), five rats in each group. In treatment groups, rats received the extract of *L. spinosa* by oral gavage (force feeding) at doses of suspension of 5, 10, 20 and 40 g/kg bw, respectively. The control group was given distilled water instead. For the first 3 hour post-exposure, the animals were observed continuously for toxicity signs including 10% loss of weight, restlessness, hunched posture, unresponsiveness and labored breathing. After 24 hour, the number of animal survivors was counted and these animals were kept to observe toxic symptoms daily for 7 days. At 7 days after gavages, all rats in each group were sacrificed. Blood samples were collected for routine hematologic and serum chemical parameters including creatinine, blood urea nitrogen, aspartate transaminase, alanine transaminase, alkaline phosphatase, cholesterol, triglycerides, total protein and albumin using automatic machines (Coulter MaxM & Beckman 6300 Chemistry analyser, Beckman, USA). Serum testosterone was determined according to the RIA procedure (Kamonpatana et al., 1979; Kamonpatana, 1986). The sperm count was determined in the right caudal epididymis. Briefly, spermatozoa were stained with eosin-nigrosin dye and evaluated using Neubauer haemocytometer. Tissue samples of lung, heart, liver, spleen, kidney, gastrointestinal tracts and testes were fixed in 10% buffered neutral formalin for routine histopathological evaluations.

Long-term reproductive efficacy test: Three groups of 6 rats were daily given the water extract of *L. spinosa* by gavage daily at doses of 5 and 20 g/kg body weight (treatment groups) and distilled water (control) for 28 days. Body weights of all rats were

noted and changes of physical and behavior were observed daily. On the twenty-eighth day, all animals were sacrificed and blood and tissue samples were collected. The blood sample was collected for hematologic, serum chemical parameters and serum testosterone evaluation. Visceral organs (lung, heart, liver, spleen, kidney and gastrointestinal tract) and reproductive organs (testis and accessory glands) were examined, weighed and fixed in 10% buffered neutral formalin. The sperm in the caudal epididymis were also counted.

Statistical analysis: Analysis of variance (ANOVA; Tukey' multiple comparison method) was used to compare the data of hematologic, serum chemical parameters, sperm count and serum testosterone levels between control and treatment groups by the SPSS statistical software for Windows, version 12. The level statistical significance value in this study was $p < 0.05$.

Results

Short term toxicity and reproductive efficacy tests revealed no mortality during the 7-day post-exposure in both control and treatment groups. No any change in general appearance was observed in both control and treatment groups during the observation period. There were no significant alterations in gross and microscopic findings of the vital and reproductive organs in both control and treatment groups. The hematological analysis showed no significant changes of hematology in the treatment groups compared to the control group (data not shown). There were no significant differences in any biochemical parameters in either the control or treatment groups of rats (Table 1). However, a significantly different ($p < 0.05$) decrease was noted in the triglyceride levels of the serum in all treatment groups compared to the control groups. For the sperm count of caudal epididymis, treated groups with the dose of 40 g/kg of the water extract showed significant increase ($p < 0.05$) after 7 days when compared with the control group (Fig 1). There were no differences in the serum testosterone levels of all treatment groups compared with the control group (Fig 2).

Table 1 Effects of *L. spinosa* on biochemical parameters after 7 days of treatment.

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)
Control	68.4±2.88	184.2±25.36	6.48±0.15	3.14±0.09	177.2±81.07	69.6±26.31	241.4±25.13	0.1±0.0	32.6±5.18	0.56±0.05
5 g/kg bw	64.6±4.10	114.0±18.12*	6.06±0.24	3.10±0.10	124.8±19.79	48.2±7.79	229.6±32.51	0.1±0.0	29.2±2.28	0.54±0.05
10 g/kg bw	66.8±2.95	132.6±14.05*	6.20±0.26	3.16±0.11	189.0±85.38	67.8±32.03	260.0±59.16	0.1±0.0	31.6±2.07	0.60±0.00
20 g/kg bw	62.6±8.26	114.2±20.23*	5.96±0.47	3.04±0.19	197.8±61.66	70.4±17.67	268.8±22.49	0.1±0.0	33.4±2.41	0.54±0.05
40 g/kg bw	66.4±2.88	135.6±38.86*	6.34±0.11	3.18±0.08	174.6±129.72	67.2±20.81	247.2±18.46	0.1±0.0	34.4±1.14	0.60±0.00

*All data are expressed as means±SE. Differences with $p < 0.05$ are considered statistically significant.

AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, BUN: blood urea nitrogen

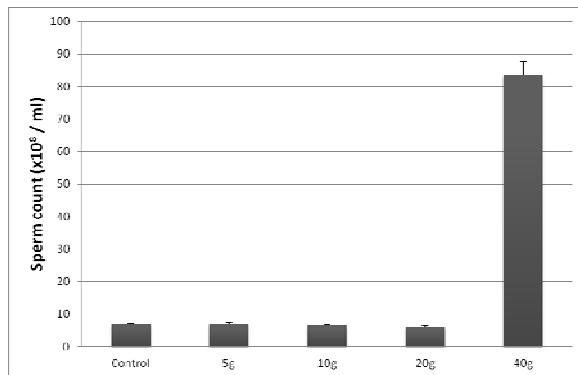


Figure 1 Effect of the *L. spinosa* on sperm count after 7 days of treatment ($\times 10^6 / \text{ml}$). *All data are expressed as means \pm SE and were compared with the ANOVA (n = 5). Differences with $p < 0.05$ are considered statistically significant.

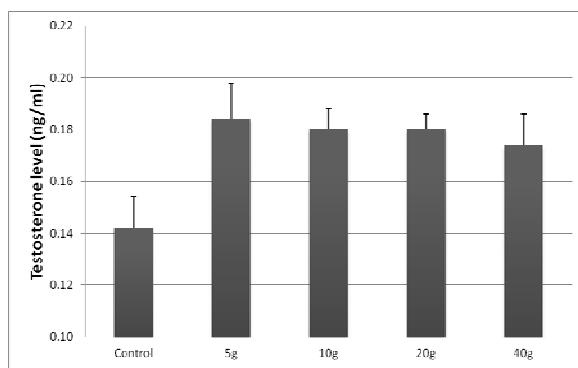


Figure 2 Effect of the *L. spinosa* on serum testosterone after 7 days of treatment. *All data are expressed as means \pm SE and were compared with the ANOVA (n = 5). Differences with $p < 0.05$ are considered statistically significant.

In subchronic period of toxicity and reproductive effect experiments, no lethality and toxic sign were recorded for any treatment and control groups during 28 days of treatment except the rats gavaged with 20 g/kg BW showed mild loss of appetite during the observation period. The 20 g/kg group showed significantly lower body weight than the control group (Table 2). There was no significant difference in the weight ratios of the vital organs between the treatment and control groups. The dose of 20 g/kg of *L. spinosa* extract caused significant elevation ($p < 0.05$) in testicular weight compared to the control group after 28 days of treatment. Moreover, the 5 g/kg treated group showed significant increase in right testicular weight compared with the control group (Table 2). No changes of the accessory gland weight were observed. No gross and histopathological changes were noted in vital and reproductive organs of all control and treatment groups. The hematological (data not shown) and biochemical analysis showed no significant changes in the treatment groups compared to the control group. However, the triglyceride level in the 20 g/kg treated group was significantly decreased in comparison to the control group (Table 3).

Table 2 Effects of *L. spinosa* on body weight gain and weight of organs after 28 days of treatment.

Group	Body Weight	Liver	Spleen	Brain	Lung	Rt. adrenal gland	Lt. adrenal gland	Thymus	Rt. Kidney	Lt. Kidney	Rt. Testis	Lt. Testis	Accessory organs
control	455.18 \pm 23.9	3.58 \pm 0.21	0.212 \pm 0.01	0.451 \pm 0.08	0.414 \pm 0.08	0.002 \pm 0.0	0.005 \pm 0.01	0.124 \pm 0.03	0.311 \pm 0.03	0.309 \pm 0.02	0.621 \pm 0.03	0.600 \pm 0.04	0.575 \pm 0.14
5 g/kg	411.50 \pm 15.5	3.40 \pm 0.11	0.203 \pm 0.02	0.451 \pm 0.03	0.381 \pm 0.03	0.005 \pm 0.0	0.003 \pm 0.01	0.103 \pm 0.01	0.332 \pm 0.02	0.334 \pm 0.023	0.699 \pm 0.03	0.680 \pm 0.01*	0.684 \pm 0.09
20 g/kg	374.29 \pm 36.4*	3.39 \pm 0.30	0.207 \pm 0.02	0.527 \pm 0.06	0.491 \pm 0.13	0.006 \pm 0.0	0.007 \pm 0.0	0.085 \pm 0.03	0.345 \pm 0.03	0.341 \pm 0.02	0.708 \pm 0.02	0.713 \pm 0.04*	0.595 \pm 0.10

*All data are expressed as means \pm SD and were compared with the ANOVA (n=6). Differences with $P<0.05$ are considered statistically significant.

Table 3 Effects of *L. spinosa* on biochemical parameters after 28 days of treatment.

Group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	BUN (mg/dl)	Creatinine (mg/dl)
control	73.00±3.61	197.00±35.68	5.87±0.29	3.53±0.06	227.33±94.30	121.00±115.18	145.33±32.62	25.00±2.65	0.60±0
5 g/kg	68.17±4.93	150.50±39.28	5.60±0.25	3.52±0.13	140.17±69.45	42.83±13.98	128.00±28.28	20.67±7.17	0.57±0.04
20 g/kg	68.33±4.58	94.33±22.24*	5.62±0.29	3.50±0.17	197.83±38.45	56.83±6.31	130.50±20.40	20.83±0.52	0.58±0.05

*All data are expressed as means±SD. Differences with $p < 0.05$ are considered statistically significant.

AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, BUN: blood urea nitrogen.

In the caudal epididymal sperm count, the 5 g/kg treated group gave significantly higher number than the control group (Fig 3). However, no significant differences in the serum testosterone level were observed between the control and treatment groups (Fig 4).

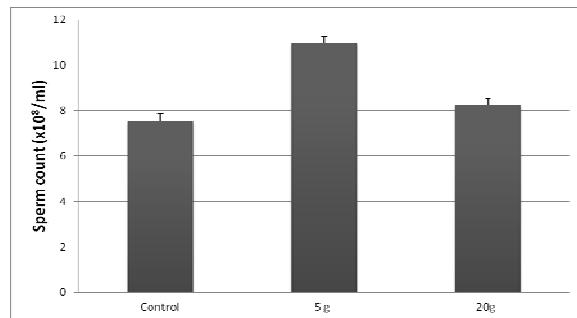


Figure 3 Effect of the *L. spinosa* on sperm count after 28 days of treatment ($\times 10^6$ /ml). *All data are expressed as means±SE and were compared with the ANOVA ($n = 6$). Differences with $p < 0.05$ are considered statistically significant.

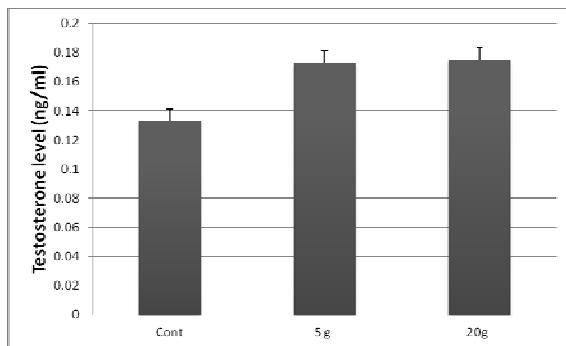


Figure 4 Effect of the *L. spinosa* on serum testosterone after 28 days of treatment. *All data are expressed as means±SE and were compared with the ANOVA ($n = 6$). Differences with $p < 0.05$ are considered statistically significant.

Discussion

In this study, there were no changes in clinical signs, hematology, clinical chemistry and pathology in different dosage of treated groups in acute and subchronic periods. These results indicated that the aqueous ethanol extract of rhizomes of *Lasia spinosa* Thw. could be categorized as the low toxic substance.

The hematological status at 7 or 28 days of oral administration of *L. spinosa* extract in the rats was also determined. RBC and WBC analysis showed no significant difference between the control and

treatment groups. For the results of biochemical analyses, the triglyceride values in the treatment rats were significantly decreased compared to the control rats. However, no significant changes in the other biochemical parameters were found. These results indicated that the oral administration of *L. spinosa* extract could decrease the serum triglyceride in rats. The mechanism of *L. spinosa* extract to decrease the blood triglyceride is still not understood.

The association between the oral administration of *L. spinosa* extract and the reproductive effects was revealed in the recent study. In the short term study, the 40 g/kg treated group showed significantly higher sperm count than the control group. However, no changes in serum testosterone were found. The failure of the extract of *L. spinosa* to increase the serum testosterone in the study could be explained by negative feedback mechanisms in animals that are capable of hormonal balance to inhibit the release of testosterone when the body has high level of hormone (Manosroi et al., 2006).

The subchronic term study results indicated that *L. spinosa* might have a beneficial effect to improve the function of male reproductive organs in rats. These data were confirmed by the evidence of the increase in testicular weight and sperm count. The increase in the absolute weight of the testis could be due to the elevation of androgen biosynthesis leading to the increase in serum testosterone levels. Testosterone plays an important role in the proliferation and hyperplasia of Sertoli cells and Leydig cells that can increase the size of the testis (Lincoln, 1979; Manosroi et al., 2006). Testosterone is also involved in the spermatogenesis and the growth of development of testes and male accessory reproductive glands. Although the testosterone level of the treated animals in our study was not significantly different from the control animals, the increased spermatogenic activities as evidence of high numbers of sperm count and increase in testicular weight were observed in the treated animals. These results implicated that oral administration of rhizome of *L. spinosa* could be used to improve sperm number.

In conclusion, the hydroethanolic extract of rhizome of *L. spinosa* did not induce any toxicological effects in the acute and subchronic term oral exposures in male rats. The use of the extract of *L. spinosa* is safe and could be used to increase male reproductive function.

Acknowledgments

This study was supported by funding from Innovation Center for Veterinary Science, Faculty of Veterinary Science, Chulalongkorn University and Chulalongkorn University Centenary Academic Development Project, 2010.

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