

Acute Effects of Mimosine Purified from *Leucaena leucocephala* on Male Reproductive System of Adult Mice

Efectos Agudos de la Mimosina Purificada de *Leucaena leucocephala*
en el Sistema Reprodutor Masculino de Ratones Adultos

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SUMMARY. This study attempted to examine the acute effect of purified mimosine extracted from *Leucaena leucocephala* on male reproductive system. Adult male mice were divided into 4 groups (n=8); control and 3 experimental groups treated with purified mimosine at different doses of 15, 30, and 60 mg/KgBW, respectively for 7 consecutive days. The morphological features and weights of body and reproductive organs including testis, epididymis plus vas deferens, and seminal vesicle were compared among groups. In addition, epididymal sperm concentration and the changes of histopathology of testicular tissues in all groups were observed. The results showed that mimosine in all doses did not affect mice body weights. However, all doses of mimosine could significantly reduce the absolute and relative weights of testis and seminal vesicle but not of epididymis plus vas deferens. Significantly, mimosine at doses of 30, and 60 mg/KgBW could decrease sperm concentration. Moreover, the seminiferous atrophy and degeneration were obviously found in mimosine treated mice as compared to the control. In conclusion, consumption of *Leucaena leucocephala* edible parts containing mimosine could damage male reproductive organs which may cause acute male subfertility or infertility.

KEY WORDS: Mimosine; *Leucaena leucocephala*; Testis; Seminal vesicle; Mice.

INTRODUCTION

The *Leucaena leucocephala* (Lamk.) de Wit (LL) has many properties such as used for human food, forage, firewood, soil erosion prevention, and high nutritive resources for cattle (Hong *et al.*, 2003; Meena Devi *et al.*, 2013). This plant's extracts have been documented to contain antioxidant activities (Benjakul *et al.*, 2013; Hassan *et al.*, 2014; Burawat *et al.*, 2016). However, it is very well known that this plant is toxic to cattle and poultry animals because it caused the retardation of growth, sub and infertility and death (Wayman *et al.*, 1970; Hammond, 1995; Anderson *et al.*, 2001). Recently, LL leave extract is shown to have adverse effect on male reproductive system of adult rats (Burawat *et al.*). Possibly, these toxic effects may result from mimosine action documented as a major toxic content in LL extract (Adeneye, 1991; Chanchay & Poosaran, 2009).

Mimosine has been reported to have inhibitory activities of many cancers, cell divisions, cell proliferations and differentiations (Wang *et al.*, 1995; Hughes & Cook,

1996; Krude, 1999). In addition, mimosine is responsible for herbicidal, insecticide, and nematicide activities (Tawata, 1990; Nguyen & Tawata, 2015). Indeed, mimosine also can arrest the cell cycle progression to inhibit cell proliferation of many cancer cells (Chung *et al.*, 2012; Park *et al.*, 2012; Bottini-Luzardo *et al.*, 2015; Fallon, 2015; Nguyen & Tawata; Kacar *et al.*, 2017). To provide the basic information to caution people about subfertility or infertility effects from acute consumption of the LL parts containing natural mimosine; therefore, this study attempted to investigate the acute effects of purified mimosine extracted from *Leucaena leucocephala* on reproductive system in male mice.

MATERIAL AND METHOD

Standard mimosine preparation. Purified L-Mimosine powders were purchased from Sigma-Aldrich, Lot#

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077K7007V, USA. To prepare a mimosine stock solution, the standard mimosine powder (1 g) was mixed with 0.1 N HCl adjusted for the pH with NaOH (pH 7).

Animals and treatments. ICR male mice (6-8 weeks) were purchased from the Animal Laboratory Unit, Faculty of Medicine, Khon Kaen University, Thailand. Then, mice were housed in plastic cages with wood chip bedding under a 12 h light/dark cycle at room temperature. This study was duly approved by the Animal Ethics Committee of KKU, based on the Ethics of Animal Experimentation of the National Research Council of Thailand (ref. No.0514.1.12.2/93). For treatment regime, male mice were randomly divided into 4 groups (n =8); in control group, mice were injected vehicle solution (0.1 N HCl, pH 7) and for 3 experimental groups (mimosine 15, 30, and 60), animals were injected (i.p.) with mimosine at 15, 30, and 60 mg/KgBW, respectively for 7 consecutive days. Mice were daily weighed throughout the experimental period. At the end of experiment, all mice were euthanized and sacrificed to collect and testis, epididymis plus vas deferens, and seminal vesicles.

Organ weight analysis and gross morphology. The testis, epididymis plus vas deferens, and seminal vesicles from control and experimental animals were collected. The fat pads surrounding such organs were gently removed before weighing as absolute organ weight. Then, all reproductive organs were calculated as individual relative organ weights using a formulation of one hundred multiplied by the absolute weight of reproductive organ and divided with mice body weight (g of organ/100gBw). After weighing the reproductive organs, the representative organs of control and mimosine treated groups were grossly observed for their sizes and morphology. All organs of both groups were captured by digital camera (Nikon Coolpix S2600, Japan) to be compared for morphological changes.

Sperm concentration assay. Sperm fluid was squeezed from left caudal epididymis plus vas deferens and dipped into 1 ml of phosphate buffered saline (PBS, 37 °C, pH 7.4). Then, diluted sperm fluid was centrifuged at 5000 rpm, 25 °C, for 2 min to wash sperm and followed by sperm pellet resuspension with 1 ml of PBS. To analyze the sperm number, the sperm suspension was diluted (1:20) before placing on Neubauer counting chamber to systemically count and calculate them as sperm concentration (¥106) in triplicate examinations (Iamsaard *et al.*, 2013).

Histopathological examination. The testes were fixed in 10 % phosphate buffered formalin (pH 7.4) for 48 h. Then, the fixed tissues were routinely processed for light microscope examination using automatic tissue processing at the Pathology Department, Faculty of Medicine, Khon

Kaen University. Subsequently, the paraffinized-tissue blocks were sectioned at 5-7 µm thickness and stained by hematoxylin and eosin. The stained-tissue slides were dehydrated, cleared, and mounted before observation under light microscope. The histological sections were captured by Nikon light ECLIPSE E200 microscope equipped with a DXM1200 digital camera to observe the histopathological changes of seminiferous tubules and interstitial tissues between the mimosine and control groups.

Statistical analysis. All data are expressed as mean± standard deviation (S.D.). The one way analysis of variance (ANOVA) was performed to examine the significant differences among sets of data using SPSS statistics 19.0 software (IBM SPSS Statistics). P<0.05 was considered for significant difference.

RESULTS

Effect of mimosine on body weight. After injection of purified mimosine for 7 consecutive days, the results showed that the interval body weight of animals treated with mimosine (15, 30, or 60 mg/KgBW) were not significantly different from that of control mice (Fig. 1). Notably, the ranges of averaged body weight in control and different mimosine-treated groups were approximately 30-35 grams as shown in Figure 1.

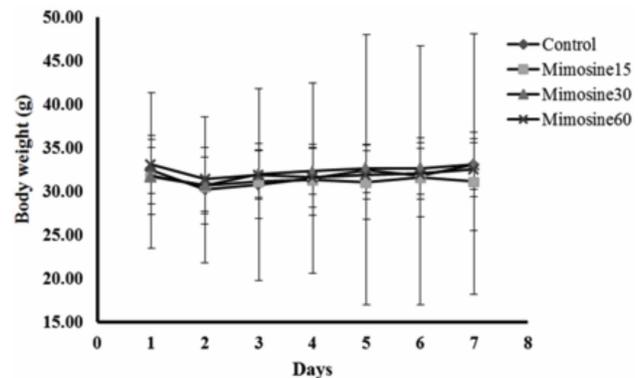


Fig. 1. Comparisons of daily body weights in 7 consecutive days of control and experimental groups (treated with 15, 30, and 60 mg/KgBW, respectively). Each data point represented as means ± S.D. (n = 8)

Effects of mimosine on reproductive morphology. The comparisons of gross morphological aspects of testis, epididymis plus vas deferens, and seminal vesicle among control, 15, 30, and 60 mg/kgBw of mimosine treated groups were demonstrated in Figure 2 (A, B, and C, respectively).

The results showed that testis and epididymis plus vas deferens in mimosine treated mice in 3 different doses were not obviously seen to be different from each other as compared to those organs of control group (Fig. 2 A, B). In contrast, the gross morphology of seminal vesicle of mice treated with only a dose of 60 mg/ KgBw was obviously smaller

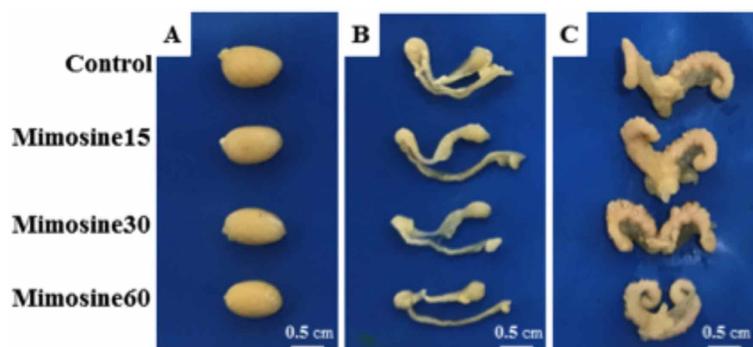


Fig. 2. Representative gross structures of testis (A), epididymis plus vas deferens (B), and seminal vesicles (C) of control and mimosine-treated mice at doses of 15, 30, or 60 mg/KgBw, respectively.

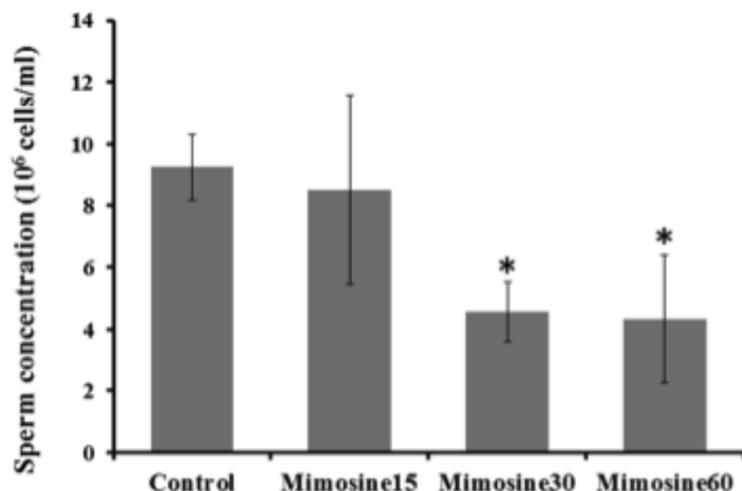


Fig. 3. Sperm concentration of control and mimosine-treated mice (15, 30, or 60 mg/KgBw). Each data point represented as means \pm S.D. (n = 8). * Significant difference ($p < 0.05$) compared to the control.

than that of control and two lower doses of mimosine (15 and 30 mg/KgBw) shown in Figure 2C.

Effects of mimosine on male reproductive organ weights. The weights of testis, epididymis plus vas deferens, and seminal vesicle of control and mimosine treated groups were quantified as absolute and relative organ weights shown in Table I. The absolute weight of testis in all mimosine treated groups were significantly decreased as compared to the control ($p < 0.05$), but only mimosine groups of 30 and 60 mg/ KgBw had significantly reductions of relative organ weight (Table I). Corroborated with gross structure results (Fig. 2), all weights of epididymis plus vas deferens among control and mimosine treated groups were not significantly different from each other ($p > 0.05$). Interestingly, both absolute and relative weights of seminal vesicles in all three mimosine treated groups were significantly decreased ($p < 0.05$) as compared to that of control mice.

Effects of mimosine on sperm concentration. To examine the acute effect of purified mimosine on sperm production, the caudal epididymal sperm were counted and calculated as sperm concentration (Fig. 3). The results revealed that mimosine at doses of 30 and 60 mg/KgBw could significantly reduce ($p < 0.05$) sperm concentration as compared to the control or low dose mimosine group (15 mg/KgBw) as shown in Figure 3.

Acute effects of mimosine on testicular histology. The representative testicular histology, stained by basic hematoxyline and eosin dyes, of control and mimosine groups are shown in

Table I. Absolute and relative weights of testis, epididymis plus vas deferens, and seminal vesicles in control and experimental mice treated with 15, 30, and 60 mg/KgBw of purified mimosine.

| Reproductive organ weights | Control | Mimosine15 | Mimosine30 | Mimosine60 |
|-------------------------------------|-------------------|--------------------|--------------------|--------------------|
| Testis | | | | |
| Absolute weight (g) | 0.2938 \pm 0.01 | 0.2499 \pm 0.03* | 0.2584 \pm 0.01* | 0.2670 \pm 0.01* |
| Relative weight (g) | 0.8024 \pm 0.12 | 0.8024 \pm 0.12 | 0.8488 \pm 0.11* | 0.8957 \pm 0.03* |
| Epididymis plus vas deferens | | | | |
| Absolute weight (g) | 0.0767 \pm 0.01 | 0.0588 \pm 0.01 | 0.0614 \pm 0.01 | 0.0635 \pm 0.01 |
| Relative weight (g) | 0.1929 \pm 0.02 | 0.1864 \pm 0.02 | 0.2024 \pm 0.04 | 0.1996 \pm 0.05 |
| Seminal vesicles | | | | |
| Absolute weight (g) | 0.3956 \pm 0.09 | 0.2149 \pm 0.08* | 0.1921 \pm 0.08* | 0.2043 \pm 0.08* |
| Relative weight (g) | 0.9322 \pm 0.21 | 0.6945 \pm 0.27* | 0.3997 \pm 0.36* | 0.6374 \pm 0.27* |

* Significant difference ($p < 0.05$) compared to the control.

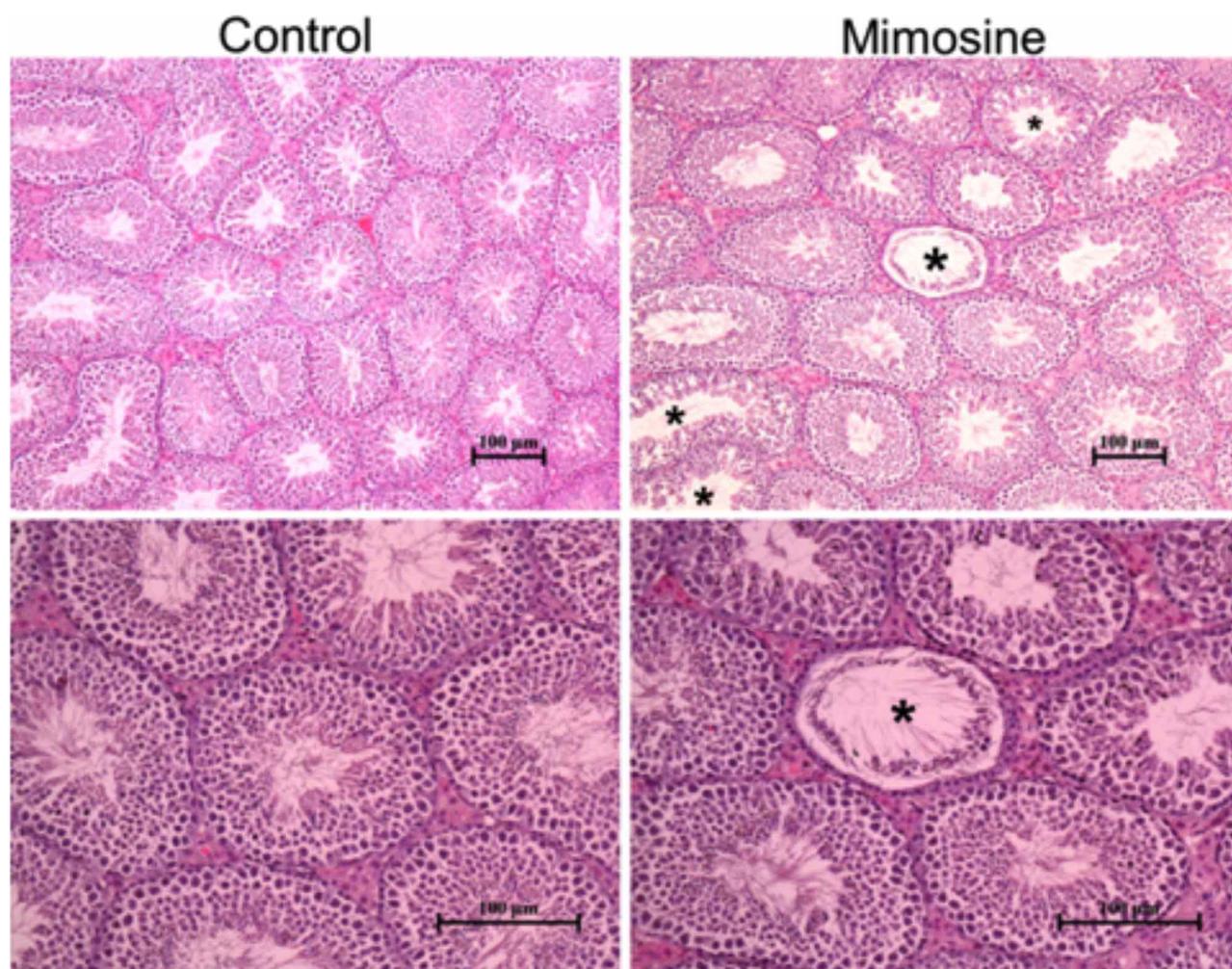


Fig. 4. Showing histopathology of representative testicular tissue of control (left panel) and mimosine-treated mice (right panel). Atrophic and germ cell degenerative seminiferous tubule (asterisks).

Figure 4. The results showed that seminiferous tubules and interstitial tissues of control mice have normal arrangement histology (Fig. 4, left panel). In contrast, it was found that mimosine in all doses could damage only seminiferous tubule approximately 10-15 % of normal tissues (Fig. 4, right panel). In histopathology of mimosine treated groups, it was observed that only slight atrophy and germ cell degeneration within tubule were notably found as compared to the control (Fig. 4). Herein, other typical testicular histopathology such as increased interstitial tissue space and dilated blood vessels were not found in all mimosine treated groups (Fig. 4).

DISCUSSION

It is very well known that all parts of *Leucaena leucocephala* (LL) tree especially leaves and seeds contain a cellular toxin, namely mimosine, which is proven to have

inhibitory activities of many cancers, cell divisions, cell proliferations and differentiations (Wang *et al.*; Hughes & Cook; Krude). The levels of mimosine content in LL depends on many factors such as locations, ages of plant, soil types, seasons, and extraction methods (Ghosh & Bandyopadhyay, 2007). Mimosine level extracted from LL grown in India was 3.33 % dry weight (Mathews & Vittal Rai, 1985) whereas of plant grown in Australia was 45 g/kg (or 4.5 % dry weight (Tangendjaja *et al.*, 1986). Additionally, the mimosine level in LL fresh leaves harvested in Malaysia examined by using High Performance Liquid Chromatography was 1.6 % wet weight (Zayed *et al.*, 2014). By colorimetric determination, mimosine level in LL leaves grown in North, Thailand was 4.4 % dry weight of total proteins (Chanchay & Poosaran). Our research group has investigated the level of mimosine content in aqueous crude extract of LL shoot tips plus young leaves cultured in Northeastern, Thailand by using Thin Layer Chromatography with method validation for the first time and we found that

its mimosine level was 17.35 % dry crude extract (Burawat, 2017). Previous study has demonstrated that LL crude extract could damage male reproductive system (Burawat *et al.*). Confirmed by specific action of mimosine, the present study has demonstrated that purified mimosine extracted from LL could directly affect reproductive organs of male mice with acute treatment. Particularly, it was found that mimosine damages testicular tissue (Fig. 4) which may affect the spermatogenesis resulting in significant decrease of epididymal sperm concentration (Fig. 3). We hypothesize that mimosine may have a major function as anti-cell proliferation of male germ cells since previous reports have demonstrated in other system about the inhibitory activities of many cancer cells, cell divisions, cell proliferations and differentiations, herbicidal, insecticide, and nematicide activities (Tawada, 1990; Wang *et al.*; Hughes & Cook; Krude; Chung *et al.*; Park *et al.*; Bottini-Luzardo *et al.*; Fallon; Nguyen & Tawata). Similar to others, the testicular histopathology, such as atrophic and germ cell degenerating seminiferous tubules, damaged by mimosine in this study was also comparable to other anti-cellular division drug demonstrated previously (Iamsaard *et al.*, 2014; Sukhorum *et al.*, 2016; Sukhorum & Iamsaard, 2017; Iamsaard *et al.*, 2017a,b; Sampanang *et al.*, 2017). In conclusion, the acute consumption of LL containing high mimosine levels must be avoided to protect the reproductive impairment in men. In parallel, further study about reversible infertility effect of mimosine should be investigated to apply this substance to be a male contraceptive.

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KANLA, P.; BURAWAT, J., ARUN, S., SAWATPANICH, T.; CHAICHUN, A. & IAMSAARD, S. Efectos agudos de la mimosina purificada de *Leucaena leucocephala* en el sistema reproductor masculino de ratones adultos. *Int. J. Morphol.*, 36(2):507-512, 2018.

RESUMEN: Este estudio intentó examinar el efecto agudo de la mimosina purificada extraída de *Leucaena leucocephala* en el sistema reproductivo masculino. Se dividieron ratones machos adultos en 4 grupos (n = 8): un grupo control y tres grupos experimentales tratados con mimosina purificada a diferentes dosis de 15, 30 y 60 mg / Kg por peso, respectivamente, durante 7 días consecutivos. Se compararon entre los grupos, las características morfológicas y el peso corporal, los órganos reproductivos, incluyendo los testículos, el epidídimo más conducto deferente y

vesícula seminal. Además, se observó la concentración de espermatozoides epididimarios y los cambios de la histopatología de los tejidos testiculares en todos los grupos. Los resultados mostraron que la mimosina no afectó los pesos corporales de los ratones. Sin embargo, todas las dosis de mimosina podrían reducir significativamente los pesos absolutos y relativos de los testículos y las glándulas seminales, pero no así del epidídimo y los conductos deferentes. La mimosina en dosis de 30 y 60 mg / Kg por peso podría disminuir significativamente la concentración de esperma. Además, se observó la atrofia y degeneración seminífera en ratones tratados con mimosina en comparación con el grupo control. En conclusión, el consumo de partes comestibles de *Leucaena leucocephala* que contienen mimosina podría dañar los órganos reproductivos masculinos, lo que puede causar subfertilidad masculina aguda o infertilidad.

PALABRAS CLAVE: Mimosina; *Leucaena leucocephala*; Testículo; Glándulas seminales; Ratón.

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