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Original Research Article

The Aprhrodisiac Effect of Ethanol Extract of *Uvaria Rufa* Blume. Bark on Male Mice (*Mus Musculus*)

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ABSTRACT

Sexual desire is essential for a harmonious sexual life, and its decrease can disrupt family stability. Globally, approximately 31% of men experience erectile dysfunction (ED). Although medications like Sildenafil are available, they pose side effects such as cyanosis, blurred vision, and hypertension. In East Nusa Tenggara, Indonesia, *Uvaria rufa* Blume has been traditionally used as an aphrodisiac. This study evaluates the acute toxicity and aphrodisiac efficacy of *U. rufa* bark extract (URE) in mice (Mus musculus). The bark was obtained using 70% ethanol, and acute toxicity was assessed via LD_{50} estimation and histological examination of liver and kidney tissues following URE administration using the fixed-dose method. Aphrodisiac efficacy was determined by measuring Leydig and Sertoli cell counts, seminiferous tubule diameter, and testicular weight after URE administration at doses of 0.425, 0.85, and 1.7 mg/20 gBW. A single 0.13 mg/20 gBW dose of Sildenafil served as a positive control. The acute toxicity test indicated an LD_{50} of 3.435 mg/kg, classifying URE as slightly toxic, while histological analysis showed no signs of necrosis, degeneration, inflammation, or cholangitis. The aphrodisiac assessment identified 0.85 mg/20 gBW as the optimal dose, significantly enhancing Leydig and Sertoli cell counts, seminiferous tubule diameter, and testicular weight. These results indicate that URE is both safe and effective as aphrodisiac, supporting its potential as a natural alternative for addressing sexual dysfunction.

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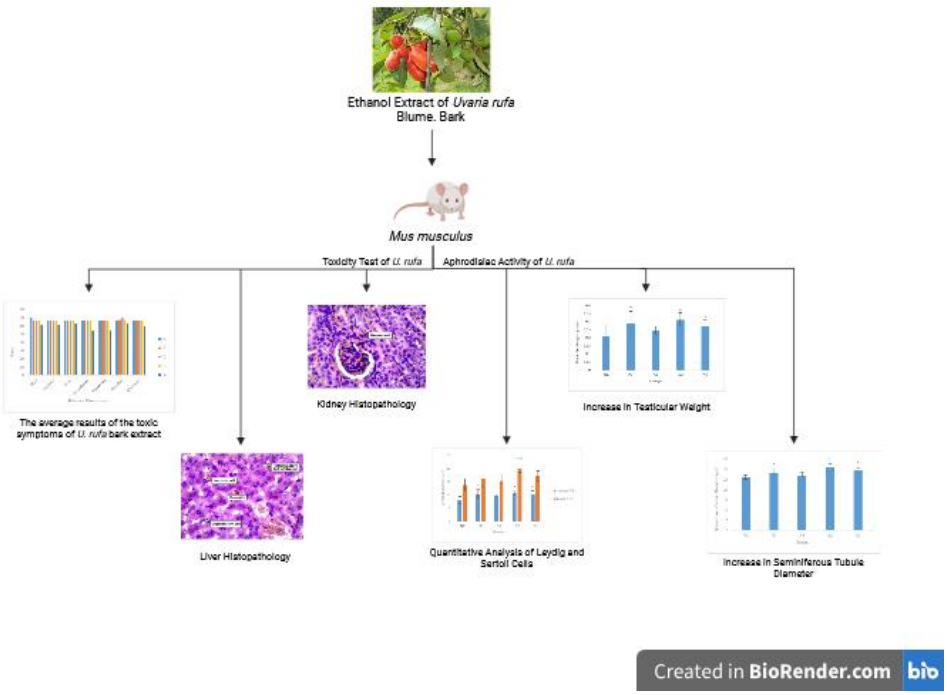
GRAPHICAL ABSTRACT



Introduction

Sexual desire is a fundamental and essential aspect for individuals in attaining a satisfying and balanced sexual experience. A decrease in sexual desire disturbs the balance of a marriage, frequently resulting from sexual disorders or dysfunction [1]. According to National Health and Social Life Survey (NHSLS) of the United States, sexual dysfunction indicates that sexual dysfunction is a disruption in sexual function, affecting about 31% of men experiencing sexual dysfunction at some stage in their lives [2]. In Indonesia, the prevalence of sexual dysfunction among men is 10% across all age groups, with over 50% found in men aged 50-70 years [3].

Male sexuality is regulated by intricate physiological mechanisms that significantly influence quality of life [4]. These mechanisms involve the neurological, circulatory, and endocrine systems, where physical, psychological, and hormonal changes can trigger sexual dysfunction. Conditions such as Parkinson's, cardiovascular disorders, and diabetes, which affect these systems, can contribute to sexual dysfunction [5]. Consequently, sexual dysfunction is a multifaceted issue that affects overall quality of life [6].

The standard treatment for sexual dysfunction typically includes the use of phosphodiesterase inhibitor medications, like sildenafil. Sildenafil functions by inhibiting phosphodiesterase isoenzymes, particularly type 5, to decrease catabolism and increase current cyclic Guanine Monophospate (cGMP) concentrations in the corpora cavernosa [7]. Nevertheless, sildenafil affects the phosphodiesterase type 6 isoenzyme in the retina, potentially causing side effects such as blurred vision, cyanopsia, increased intraocular pressure, retinal and choroidal vasodilatation, altered blood flow, and non-arteritic anterior optic neuropathy (NAION) [8]. Similarly, sildenafil encounters some challenges

such as limited availability and high cost. Therefore, there is a necessity for safer and more cost-effective treatment alternatives, such as ethnomedicine-based herbal products. A plant with potential as a natural aphrodisiac is *Uvaria rufa* (known as Lelak or Koke or Koknaba –in local languages of Timor Island, Indonesia). This plant is traditionally utilized by the people of East Nusa Tenggara Province, Indonesia to address male sexual dysfunction. The local people use a decoction made from the bark of *U. rufa* for that purpose. Phytochemical studies have shown that the ethanol extract of *U. rufa* bark contains flavonoids, alkaloids, significantly oxidized cyclohexane, rutin, isoquercetin, kaempferol 3-O-β-D-galactopyranoside, astragalin, isoquercitrin-6-acetate, benzoylated derivatives, flavonols, kaempferol, quercetin, and lignan glycosides [9].

To develop standardized herbal medicine from *U. rufa*, it is crucial to have supporting data on the safety of the extract is essential. Therefore, it is essential to conduct toxicity tests on animals to ensure the safety of aphrodisiac use, including both toxicity and activity tests. The acute toxicity test aims to detect toxic effects within a short period after administering a single or repeated dose within 24 hours [10]. The Lethal Dose 50 (LD_{50}) value is used to determine acute toxicity, defined as the dose that can statistically kill 50% of the test animal population [11]. The aphrodisiac effect of *U. rufa* was tested *in vivo* using parameters such as the number of Leydig cells, Sertoli cells, the diameter of the seminiferous tubules, and testicular weight.

Materials and Methods

Materials

U. rufa plants were obtained from Kupang, East Nusa Tenggara, Indonesia. The following chemicals were used: 70% alcohol, 70% ethanol, Tween 80, DMSO, distilled water, 80% alcohol, 96% alcohol, absolute alcohol, 10% formalin, ketamine, xylazine, hematoxylin, eosin, and xylol were purchased from Merck (Darmstadt, Germany). Male mice (M. musculus), aged 12-14 months and weighing 15-30 grams, were obtained from the Healthy Animal Clinical Laboratory, Malang, Indonesia. The study was approved by the Research Ethics Commission (Animal Care and Use Committee) at Brawijaya University, Malang, East Java, Indonesia (065-KEP-UB-2024).

Extract preparation

The bark of *U. rufa* was processed into a fine powder. Extraction was conducted using the maceration method with 70% ethanol, at a powder-to-solvent ratio of 1:15. The obtained filtrate was then concentrated using a rotary evaporator at 50 °C, with a pressure of 175 psi and a rotation speed of 70 rpm. The process was ended by evaporating the remaining solvent using an oven at 50 °C until a thick extract was obtained. This treatment obtained 93.8 grams of dry extract from 525 grams of simplicia, and a yield of 17.86%. The preparation of the stock solution was carried out using 400 mg of 70% ethanol extract from the *U. rufa* bark (URE) which was then suspended in a mixture of 0.5% DMSO and 1% Tween 80, after which it was diluted in the concentration required for toxicity and activity tests.

Toxicity test of U. rufa

Toxicity testing was conducted using the fixed dose method with doses of 5, 50, 300, and 2000 mg/kg BW to assess toxic effects [12]. Parameters observed included mouse behavior, LD50 calculations, and organ histopathology. A total of 30 mice were randomly divided into 5 treatment groups and acclimatized for 5 days [13]. The treatment groups included: (NC) Negative control receiving Tween 80 suspension and DMSO; (T1) receiving 5 mg/kg BW URE

suspension; (T2) receiving 50 mg/kg BW URE suspension; (T3) receiving 300 mg/kg BW URE suspension; and (T4) receiving 2000 mg/kg BW URE suspension. Acute toxicity adhered OECD-420 protocols [14]. Observations were made during the first 4 hours, with special attention given to the first 30 minutes, and continued at 24 hours, including evaluations of skin and fur, eyes, lethargy, convulsions, tremors, diarrhea, and death [15]. The number of deaths was recorded to determine the LD50 value, and the data were analyzed using probit statistics [16]. On the 15th day, mice were euthanized, and liver and kidney organs were removed for histopathological examination, performed in 5 fields of view at 400x magnification [17].

Aphrodisiac activity of U. rufa

Aphrodisiac activity was assessed by measuring Leydig cell count, Sertoli cell count, the diameter of the seminiferous tubules, and testicular weight. The treatment groups were: (NC) Negative control receiving 1% Tween 80 suspension in 0.5% DMSO; (PC) Positive control receiving sildenafil citrate suspension at a dose of 0.13 mg/20 g BW mice/day; (A1) receiving 0.425 mg URE suspension/20 g BW mice/day; (A2) receiving 0.85 mg URE suspension/20 g BW mice/day; and (A3) receiving 1.7 mg URE suspension/20 g BW mice/day. Each treatment was administered orally at 0.5 ml per dose for 28 days. Following treatment, mice were euthanized, and testicular organs were removed. The diameter of the seminiferous tubules was measured using a light microscope with 100x magnification (10x10) in 5 fields of view. Quantitative analysis of Leydig and Sertoli cells was performed via a light microscope with 400x magnification (40x10) in 5 fields of view.

Results and Discussion

Toxicity test results of U. rufa

Intensive observation was carried out every 30 minutes for the first 4 hours and at the 24th hour to observe toxic symptoms, such as changes in the skin (normal, irritation, and itching) and fur (normal and standing), eyes (normal, enlarged/constricted pupils, lacrimation, ptosis), lethargy (lethargy), convulsions (normal and seizures), tremors (normal and shaking), diarrhea, and death [18]. Observations were performed by three individuals for 5 minutes each to detect toxic symptoms [19]. The study results indicated that no abnormal changes were observed, allowing the main test to proceed with 6 mice per group [20]. Additional observations were carried out for 14 days to identify any deaths among the experimental animals and to document any toxic symptoms that appeared. The average results of these toxic symptoms are displayed in Figure 1.

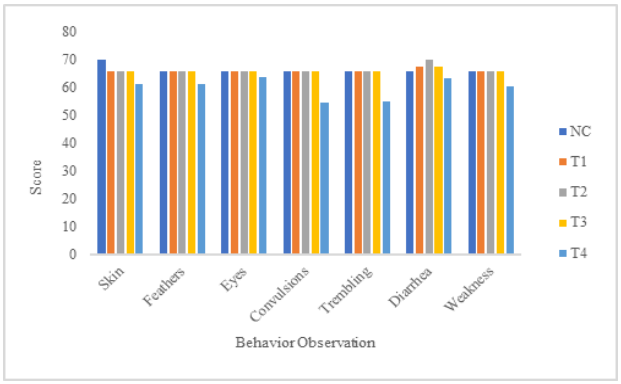


Figure 1. The average results of the toxic symptoms of *U. rufa* bark extract.

Figure 1 shows that the NC group had a stable behavioral pattern in all parameters, used as a basis for comparing the treatment groups. Along with the increase in the treatment dose, there were increasingly significant changes in several animal behavioral parameters [17]. At low doses (T1), there were minor behavioral changes, but these increased at higher doses (T4), especially affecting seizures, balance, movement, and silence. This suggests that the compound significantly influences the physiological function

of the animal. Physical traits such as skin, fur, and eyes remain stable, suggesting that the effect of the compound is stronger in functional areas than structural ones. This graph illustrates the relationship between dose and animal behavioral response. At T4, the mice behavior was not constant with death occurring on the 2nd and the 3rd days, one mouse each, so that a total of two mice died by the 14th day, this is in accordance with the fact that acute toxicity will affect behavior, appetite and drinking, hair, skin, eyes, convulsions, urination, defecation, salivation, and reflexes in experimental animals [21]. Other factors apart from high doses, like mouse resistance and errors while using the test material probe in the mouse's mouth, up to the 14-day treatment process can also lead to mortality in mice [22].

Table 1. Average weight index results for days 1-14

	NC	T1	T2	T3	T4
Mean	27.605	27.173	25.855	25.851	24.969*
SD	2.205	2.575	3.250	2.938	1.832

*NB: * Sig NC (p<0.05).*

Similarly, to assess the relationship between the development of body weight in the tested animals and toxic effects, the average daily body weight gain parameter was used due to differences in initial weights between groups. This can facilitate monitoring of weight changes over a 14-day period [23-24]. The results of the post-hoc LSD test indicated that there were no significant differences between groups ($p \geq 0.05$).

Table 2. Average weight of liver and kidney organs

Treatment Group	Average Organ Weight ± SD (grams)	
	Liver	Kidney
NC	2.16 ± 0.01	0.71 ± 0.03
T1	2.19 ± 0.01*	0.73 ± 0.01
T2	2.12 ± 0.01*	0.70 ± 0.02
T3	2.13 ± 0.01*	0.70 ± 0.03
T4	2.11 ± 0.01*	0.62 ± 0.03*

*NB: * Sig NC (p<0.05).*

The average weight index results are presented in Table 2.

Measurements of organ weights were conducted following 14 days of observation for signs of toxicity and changes in body weight in the tested animals [25]. Organs such as the liver and kidneys were surgically removed and weighed to determine their weight [19]. The results of the post-hoc LSD test revealed significant differences in nearly all data ($p < 0.05$). The average organ weight results are presented in Table 2. Histopathological examination of the livers of mice was conducted to assess necrosis, degeneration, inflammation, and cholangitis [26]. Kidney injury in the mice was marked by necrosis. Since the liver acts as a detoxification organ, its impairment may indicate the potential toxicity of a substance [27]. The results of liver damage assessments in each group of the tested animals are depicted in Figure 2. Liver histopathological scoring was conducted following the technique outlined by Knodell *et al.* [28], which involves observing a single field of view split into four sections [28].

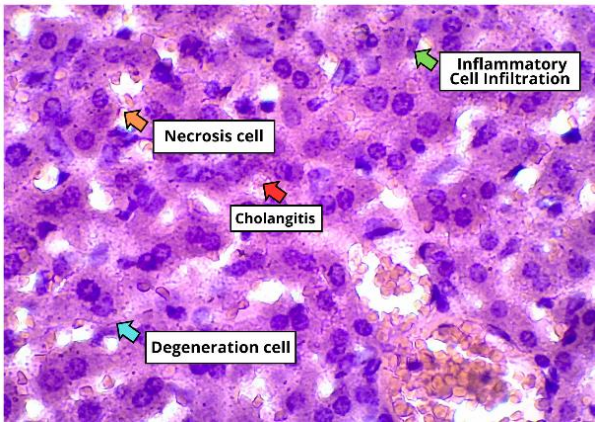


Figure 2. Liver histopathology.

At the microscopic level, hepatocyte necrosis is marked by reduced vascularization, darkened and dense chromatin (pyknosis), fragmented cell nuclei (karyorrhexis), and pale, shapeless cell nuclei (karyolysis) [29]. Hydropic degeneration, on the other hand, is a degenerative disorder

where cells expand to double their normal size. This condition, which is more serious than parenchymatous degeneration, is marked by the development water-filled vacuoles in cells lacking fat and the occurrence of clear areas in the cytoplasm [30,31]. The mean results of the observations of necrosis and degeneration are showed in Figure 3.

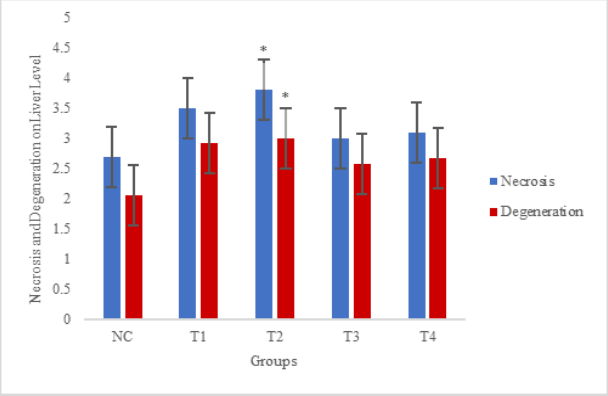


Figure 3. Necrosis level and degeneration on liver, *: Sig NC ($p<0.05$).

Figure 3 demonstrates that administration of T2 leads to the most significant necrosis and degeneration relative to the NC. In contrast, T3 exhibits results similar to NC, with the post-hoc LSD test showing no significant differences, suggesting that T3 is the ideal dose. Inflammation is a localized response to damage in tissue. Lymphocyte infiltration in the central vein occurs due to damage to endothelial cells sensitive to toxins, starting in the central vein and spreading to the portal area with extended toxic exposure [27,32]. Furthermore, cholangitis refers to inflammation of the bile ducts [33]. The average observation results of cell infiltration, inflammation, and cholangitis are illustrated in Figure 4.

Figure 4 illustrates the extent of inflammatory cell infiltration and cholangitis in the livers of mice following treatment with different doses of URE. The T2 dosage led to marked infiltration of inflammatory cell and notabe cholangitis effects in the liver tissue.

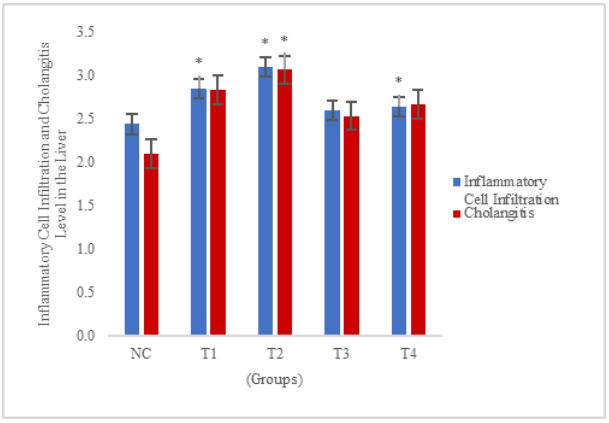


Figure 4. Inflammatory cell infiltration and cholangitis level in liver, *: Sig NC ($p<0.05$).

In contrast, administrating T3 results similar to the NC, and the post-hoc LSD test revealed no significant differences, suggesting that T3 is the ideal dose. The kidneys function to eliminate metabolic products and toxins via urine, and they are involved in filtration, reabsorption, secretion, maintaining fluid balance, and producing renin, erythropoietin, and prostaglandins [34]. Histopathological examinations of the kidneys in male mice exhibited necrotic damage from URE treatment, as shown in Figure 5.

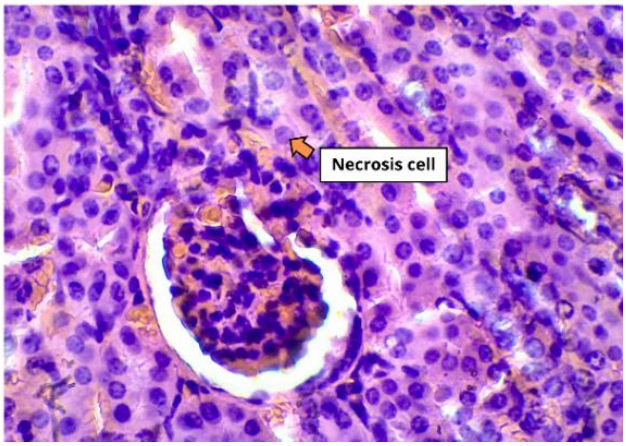


Figure 5. Kidney histopathology.

Kidney histopathological scoring was conducted by observing one field of view split into four sections, with scores ranging from 0 to 3 based on the level of necrotic damage [34]. The average

results of necrosis examinations across five fields of view are indicated in Figure 6.

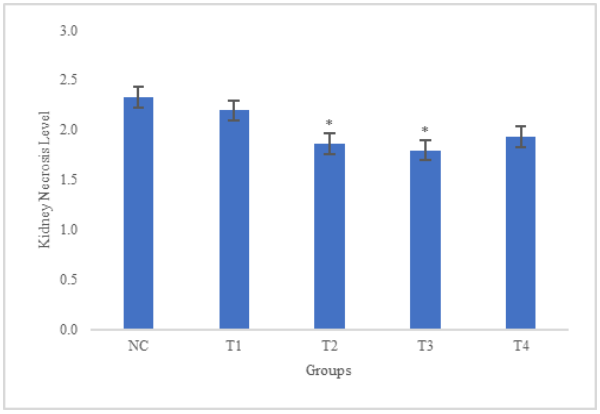


Figure 6. Kidney necrosis level, *: Sig NC (p<0.05).

Figure 6 reveals that Negative Control group achieved the highest score, while group Group 3 recorded the lowest score, reflecting the most significant reduction in kidney damage and demonstrating the optimal protective effect ($p < 0.05$). Other doses also showed a decrease in kidney damage compared to the Negative Control. The LD_{50} was determined through probit analysis, with the LD_{50} for the acute toxicity test of 70% ethanol extract of *U. rufa* bark calculated at 3,435 mg/kg, placing it as the mildly toxic category (2000-5000 mg/kg) [13].

Aphrodisiac activity test results of U. rufa

In the aphrodisiac activity test, the number of Leydig cells was assessed to evaluate androgen synthesis normalcy, as Leydig cells are the main location of androgen synthesis in mammals [35]. Leydig cells are located in the testicular interstitium between the seminiferous tubules [36]. The average results of observations for Leydig cells and Sertoli cells are illustrated in Figure 7.

The results of the post-hoc analysis in Figure 7 show that the number of both Leydig and sertoli cells increased after administration of PC in the treatment group compared to NC, with the highest A2 presenting a significant difference (p<0.05) from NC.

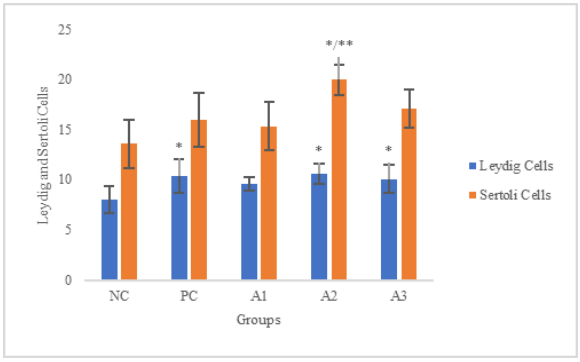


Figure 7. Quantitative analysis of Leydig and sertoli cells, *: Sig NC (p<0.05); **: Sig PC (p<0.05).

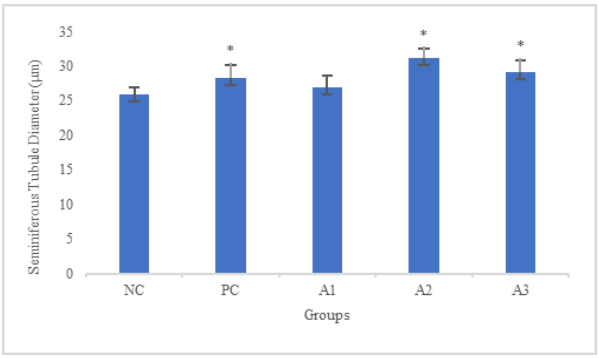


Figure 8. Increase in seminiferous tubule diameter, *: Sig NC (p<0.05).

The primary component of the testicular mass is the seminiferous tubules which function to produce around 30 million spermatozoa daily throughout the production period [37]. The diameter of the seminiferous tubules increased after administration of PC, A2 and A3, showing a significant difference (p<0.05) with the NC group. The average observation results for the diameter of the seminiferous tubules are shown in Figure 8. The results of post-hoc analysis A2 have the most optimal diameter of the seminiferous tubules. The testes are male genital organs which serve as a site for the synthesis of androgen hormones, particularly testosterone [38]. Other studies indicate that testicle weight is closely linked to sperm production [39].

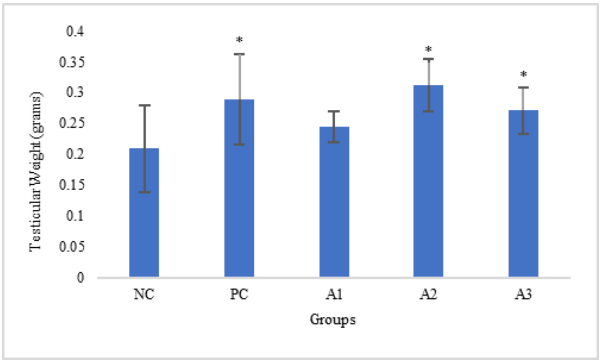


Figure 9. Increase in Testicular Weight, *: Sig NC ($p<0.05$).

Testosterone is the most important steroid and androgen hormone for libido and spermatogenesis in men [40]. Post-hoc analysis results in Figure 9 show that the average testicular weight after administration of PC and the A2 treatment group was the highest and demonstrate a significant difference ($p<0.05$) compared to the NC group. Aphrodisiacs enhance sexual desire and/or pleasure and can stimulate libido. Plant compounds with potential aphrodisiac properties include steroid saponins, alkaloids, and flavonoids [41]. *Uvaria rufa* has flavonoids [9]. The aphrodisiac activity of the plant may be associated with its antioxidant properties. Flavonoids are amphipathic substances that can pass through lipid bilayer membranes. This trait may enable them to safeguard the acrosome and sperm membrane from oxidative damage. Therefore, they play a crucial role in ensuring the necessary sperm response for successful conception [42]. Spermatogenesis occurs in the seminiferous tubules of the testes in multiple stages, requiring autocrine, paracrine, and endocrine stimulation regulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [43]. LH and FSH concentrations influence androgen hormone production. The disrupted FSH secretion can reduce Sertoli cells function to form Androgen Binding Protein (ABP), while the disrupted LH secretion can reduce Leydig cells function to form testosterone. ABP binds the testosterone to surround spermatogenic cells for maintaining the normal spermatogenesis activity. If testosterone is decreased, the spermatogenic cells production will also decrease in number [44]. In this treatment, the number of Leydig cells and Sertoli cells in A2 exhibited the highest value among the URE administration groups, showing a significant difference from group NC, this indicated that A2 produced the most testosterone. This is in line with the diameter of the seminiferous tubules and the weight of the testes in A2 which were larger than those in other groups. Flavonoids can interact with luteinizing hormone receptors, a component of the G protein-coupled membrane receptor family. This interaction enhances the expression of the steroidogenic acute regulatory protein (StAR) gene, stimulating androgen production in Leydig cells [35]. In addition, flavonoids enhance steroidogenesis by increasing cholesterol import into mitochondria, which boosts StAR levels in Leydig cells. In Leydig cells, as in other steroidogenic tissues, cholesterol is the primary substrate for steroid synthesis [35]. Along with FSH and LH, testosterone is necessary for spermatozoa to survive its life in the epididymis. Testosterone is also involved in glucose uptake, which is metabolized in the mitochondria to generate ATP, the primary energy source for spermatozoa to maintain its motility, activity, and life [44]. The aphrodisiac effects of flavonoids are also affected by their ability to inhibit thromboxane A2 receptors. This inhibition reduces the activity of the proinflammatory transcription factor nuclear factor-kappaB (NF-κB), thereby decreasing cyclooxygenase-2 (COX-2) expression and modulating StAR expression in Leydig cells. Moreover, flavonoids stimulate human chorionic gonadotropin (HCG) in Leydig cells, which can increase testosterone production [35]. Flavonoids bind to guanylate cyclase receptors, stimulating the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway.

Activating this pathway enhances StAR gene expression and prevents reduction in testosterone levels. Furthermore, the NO/cGMP pathway inhibits phosphodiesterase 5 (PDE5) in Leydig cells, which activates the steroidogenic pathway and results in testosterone secretion [4]. This mechanism is similar to the action of sildenafil as a positive control, by inhibiting phosphodiesterase 5 (PDE5) and increasing testosterone levels [45,46].

Conclusion

The 70% ethanol extract from *U. rufa* bark demonstrates low toxicity in mice, with an LD_{50} value of 3,435 mg/kg, which is consistent with both quantitative and qualitative findings. In aphrodisiac activity testing, the administration of A2 (0.85 mg/20 g BW mice/day) proved to be the optimal dose, as it significantly increased the number of Leydig cells, Sertoli cells, the diameter of seminiferous tubules, and testicular weight. URE has demonstrated safety and efficacy as an aphrodisiac, suggesting its potential for being developed as an alternative therapy for sexual dysfunction.

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Conflict of Interest

The authors declared that there was no conflict of interest in this study.

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