RIPE PAPAYA (CARICA PAPAYA) REDUCES UTERINE LENGTH AND WEIGHT IN MICE (MUS MUSCULUS L)

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Abstract. Unripe or green papaya is one of the foods that should be avoided during pregnancy due to its potential to cause fetal abnormalities and/or miscarriage. Papaya contains papain, polysaccharides, alkaloids, saponins, and flavonoids, which are suspected to interfere with the hypothalamic-pituitary axis, disrupting GnRH secretion and subsequently affecting follicular formation, development, and maturation. This study aimed to investigate the effect of ripe papaya (Carica papaya) fruit juice administration on uterine characteristics in mice (Mus musculus). A total of 12 adult female mice were randomly divided into four groups and administered ripe papaya juice orally (gavage) at doses of 0% (control), 20%, 40%, and 80% for ten consecutive days. Following treatment, the mice were sacrificed for uterine collection. Data were analyzed using ANOVA followed by Duncans post hoc test. The results indicated that the administration of ripe papaya juice significantly reduced both left and right uterine lengths and lean wet weights at all doses. The reduction in uterine length on both sides suggests that active compounds in papaya, such as the enzyme papain, may influence cellular proliferation processes within uterine tissue.
Keywords: *ripe papaya juice, papain, uterus, fetal*

Introduction

Papaya (Carica papaya L.) is a tropical fruit that originates from plants in the family Caricaceae. This plant was first discovered in Central America, the West Indies, and around Mexico and Costa Rica (Al-Ikhsan et al., 2024). Papaya can grow well in a variety of geographic conditions, including tropical and subtropical regions, with varying levels of rainfall, and in both lowland and highland areas (Parera et al., 2022). The fruit is rich in nutrients such as provitamin A, provitamin C, vitamin B, and lycopene, as well as essential minerals and fibre, which are beneficial for human health (Hidayati and Ruhayani, 2021). In addition to its fruit, papaya leaves also contain bioactive compounds such as alkaloids, flavonoids, saponins, phenols, steroids, and tannins, which have anti-fatigue properties (Candra et al., 2024; Santi et al., 2023). Almost all parts of the papaya plantincluding the leaves, stems, roots, and fruitare utilized for various purposes (Jiménez et al., 2024; Koul et al., 2022). Papaya latex, commonly known as papain, has several industrial applications, including beer clarification, meat tenderization, leather tanning, and use in the pharmaceutical and cosmetic industries (Nekoueinaeini et al., 2024; Marwah et al., 2023). According to David Troncoso et al. (2022), papain is a protease enzyme widely used in industries such as food processing, detergent production, and photography and as an active ingredient in skin cleansers. However, papain can cause skin irritation upon contact, likely due to its proteolytic activity (Maliha et al., 2024; Stanciu and Sassville, 2015).

Papain is also known to exhibit teratogenic and abortifacient effects (Campos et al., 2023; Adigwe et al., 2012; Adebiyi et al., 2002). It can stimulate uterine contractions due to its action that mimics prostaglandins and oxytocin, thereby potentially inducing

labour and endangering both the mother and fetus (Suryani and Harlita, 2025; Praveena et al., 2017). Research by Jaiswal et al. (2010) demonstrated that papain from papaya latex possesses anti-implantation activity and can induce embryotoxicity in Wistar rats when administered orally. Anuar et al. (2008) further reported that unripe papaya may lead to miscarriage in pregnant women, especially among those with higher susceptibility, due to the elevated latex content compared to ripe papaya. The uterus is a female reproductive organ that serves as the site of fertilized ovum implantation and fetal development. In mice, the uterus has a duplex structure characterized by two separate uterine horns (Harlita et al., 2015). Monitoring the estrous cycle in experimental animals is a valuable indicator for evaluating the integrity of the hypothalamic-pituitary-ovarian axis and the functional status of the female reproductive system (Auta and Hassan, 2016). It is also a valuable method for assessing the effects of drugs and chemicals on reproductive function (Byers et al., 2012).

Lack of early pregnancy awareness and insufficient attention to proper nutrition in the first trimester can pose significant risks to both the mother and the fetus. During this critical stage of fetal development, the body undergoes organogenesis, which is highly sensitive to external factors, including dietary intake. Suppose a woman is unaware of her pregnancy. In that case, she may inadvertently consume high-risk foods, such as raw or unregulated products, including papaya containing latex and papain, that can trigger uterine contractions and increase the risk of miscarriage. Inadequate nutrition or consumption of harmful substances during this period may disrupt fetal development, potentially resulting in congenital abnormalities or mental and physical defects in newborns. Furthermore, delays in accessing appropriate medical care or undergoing routine pregnancy check-ups may prevent early detection of fetal conditions or implementation of preventive measures, thereby increasing the health risks for both mother and child. This study aims to evaluate the effects of ripe papaya (Carica papaya) fruit juice administration on the uterine characteristics of female mice (Mus musculus L.).

Materials and Methods

This study employed a completely randomized design (CRD) with four treatment groups and three replications. The treatments included a control group (no juice administration) and groups receiving 20%, 40%, and 80% concentrations of ripe papaya juice, administered orally from day 6 to day 15 of the estrous cycle. The equipment used in this study included mouse cages, surgical knives, dissection boards, plastic bowls, magnifying glasses (loupes), analytical balances, tweezers, blenders, and cameras. The materials used consisted of adult female mice, ripe papaya fruits, 0.9% NaCl solution, 70% ethanol, BR-type mouse feed, straw, pregnant mare serum gonadotropin (PMSG), and human chorionic gonadotropin (hCG). This study was conducted in several stages: preparation of ripe papaya juice, preparation of animal models, treatment and observation phase, and uterine analysis. The observed variables included the uteruss lean wet weight, left uterine length, right uterine length, and total uterine length. Ripe papaya fruits were peeled, washed, and diced into 2×2 cm pieces, then blended into a smooth consistency. The following concentrations were prepared: 0% (control): distilled water only, 20%: 2 mL juice + 8 mL distilled water, 40%: 4 mL juice + 6 mL distilled water and 80%: 8 mL juice + 2 mL distilled water.

Female mice were housed in plastic cages with wire-mesh lids and rice husk bedding and fed BR-type pellets. To induce superovulation, PMSG was administered intraperitoneally on the first day, followed by hCG on the third day. After hormonal induction, the females were mated with males using the one-mating method. Each female was tagged and later housed individually, then assigned to the appropriate treatment group. Sixteen adult female mice, approximately 2 months old, were randomly divided into four groups (four mice per group): (1) Group I (Control): No treatment; (2) Group II: 20% papaya juice; (3) Group III: 40% papaya juice; and (4) Group IV: 80% papaya juice. Ripe papaya juice was administered via oral gavage at a dose of 0.2 mL/mouse/day for 10 consecutive days. After treatment, the mice were weighed and sacrificed. Necropsy was performed by applying cervical dislocation, followed by dissection to observe the research variables, including the lean wet weight of the uterus, left uterine length, right uterine length, and total uterine length. Before analysis, each mouse was weighed, followed by a necropsy to extract the uterus. The uterine horns were measured for length and then subjected to fat extraction using ethanol (24 hours) and ether (twice, 24 hours each) to obtain the uteruss lean, wet weight. To determine the effects of ripe papaya juice on uterine characteristics, data from each treatment group were analyzed using Analysis of Variance (ANOVA), followed by Duncan's multiple range test at a 95% confidence level (α=0.05), using SAS statistical software.

Results and Discussion

Left and right uterine lengths in mice

The measurements of the left and right uterine lengths are presented in *Table 1*. As shown in *Table 1*, the administration of ripe papaya juice at a 20% concentration resulted in an average left uterine length of 1.00 cm and a right uterine length of 0.97 cm. At 40% concentration, the left uterine length was 0.98 cm, and the right was 1.01 cm. Meanwhile, at 80%, the left uterine length was 0.90 cm and the right 0.92 cm. Overall, mice treated with 20%, 40%, and 80% papaya juice concentrations exhibited shorter uterine lengths compared to the control group (0%), in which the left and right uterine lengths were 1.03 cm and 1.07 cm, respectively. According to the results of the Analysis of Variance (ANOVA), the administration of ripe papaya juice significantly affected the left uterine length, as indicated by a significance value (P-value)<0.05. However, the treatment did not significantly influence the right uterine length, as shown by a P-value>0.05. Duncans post hoc test revealed that the left uterine length in the control group (0%) did not significantly differ from that in the 20% and 40% treatment groups. However, the 0% group differed substantially from the 80% group. Additionally, the 20% and 40% groups did not significantly differ from each other, but both differed from the 80% group. These results are illustrated in *Figure 1*.

Table 1. *Left uterine length, right uterine length, and total uterine length in mice.*

Treatment	Uterine length (cm) ± SD	
	Left	Right
0% (Control)	1,03±0,003[a]	1,07±0,003[a]
20%	1,00±0,003[a]	0,97±0,037[a]
40%	0,98±0,035[a]	1,01±0,00[a]
80%	0,90±0,03[b]	0,92±0,008[b]

Note: Superscript letters in the same column indicate no significant difference (P < 0.05).

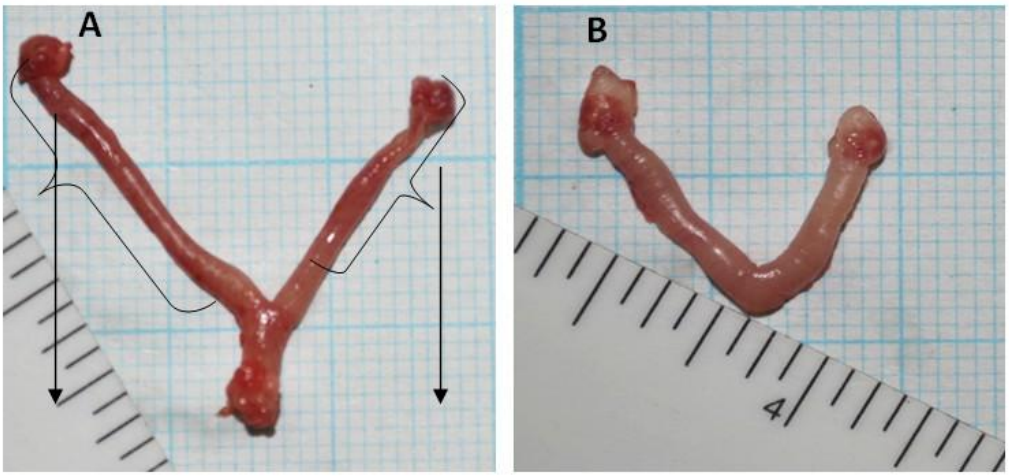

Figure 1. *Uterus of Mus musculus: (A) Uterus from mice not treated with ripe papaya juice (control group); and (B) Uterus from mice treated with ripe papaya juice.*

Based on the research findings (*Table 1*), it is evident that the administration of ripe papaya juice at doses of 20%, 40%, and 80% resulted in shorter uterine lengths compared to the 0% dose. The reduction in uterine length at the 20%, 40%, and 80% doses can be associated with the presence of papain compounds in the papaya fruit. Papain is a proteolytic enzyme that can break down proteins and influence various biochemical processes in the body (Babalola et al., 2024). Papain is believed to interfere with the proliferation of cells in the uterus (Memudu and Oluwole, 2021). According to Ruijtenberg and Van Den Heuvel (2016), cell proliferation is a crucial process for the division and growth of cells that is essential for tissue formation and development. The reduction in cell proliferation in the uterus due to papain may inhibit the development of the endometrial and myometrial tissues, Ih are Involved In determining the length and volume of the uterus (Jaiswal et al., 2010). As a result, a reduction in uterine length occurs, which can be observed in the higher-dose papaya juice treatment groups. Furthermore, the papain enzyme may play a role in autolysis, which is the breakdown of cells via enzymatic mechanisms. Papain can degrade structural proteins within cells, leading to tissue damage and potentially causing atrophy. Uterine atrophy is the reduction in organ size that can occur due to a decrease in cell number or cell size (Choudhary et al., 2025; Novinec and Lenarčič, 2013). The reduction in uterine length observed in the papaya juice treatment groups suggests a toxic effect or inhibition on the processes of cell formation and development within the uterus. Additionally, papain in papaya is known to influence the body's hormonal cycle, which regulates the enlargement and development of reproductive organs. The natural compounds in papaya, including papain, may disturb the hormonal balance, particularly the hormones estrogen and progesterone, which are essential for the reproductive cycle and uterine development. Changes in these hormone levels can lead to alterations in the structure and size of the uterus, ultimately resulting in a decrease in uterine length (Iipinge, 2019; Kusemiju, 2008).

Lean Wet Weight (LWW) of the uterus

The mean values of the uterine lean wet weight (LWW) are presented in *Table 2*. The results presented in *Table 2* indicate that, in the control group (0%), the lean wet weight (LWW) of the mouse uterus was recorded at 0.43 g. In contrast, the groups treated with ripe papaya juice exhibited significantly lower LWW. At the 20% dose, the LWW was recorded at 0.13 g, at the 40% dose it was 0.12 g, and at the 80% dose, it reached 0.09 g. Based on the Analysis of Variance (ANOVA), the significance value (sig) was found to be <0.05, indicating that the administration of ripe papaya juice had a significant effect on the lean wet weight (LWW) of the mouse uterus. The results of the Duncans multiple range test revealed that the LWW of the uterus in the control group (0%) did not differ significantly from the 20% and 40% treatments, but it was significantly different from the 80% treatment group. Furthermore, the LWW in the 20% treatment group did not differ significantly from the 40% group, but it was significantly different from the 80% treatment group. The lean, wet weight of the uterus refers to the uterus from which fat has been removed, leaving the clean weight of the organ while still containing water. In this study, the administration of ripe papaya juice at doses of 20%, 40%, and 80% resulted in a reduction in the lean wet weight of the mouse uterus. This decrease in weight is linked to the role of the papain enzyme found in papaya. Papain, as a proteolytic enzyme, is capable of breaking down proteins and potentially disrupting the growth and development of cells in the uterus, leading to an overall reduction in uterine weight (Jamal et al., 2025; Gulia et al., 2023; Malek et al., 2016).

***Table 2**. Lean wet weight of the uterus.*

Parameter	Treatment ($\bar{x}$ ± SD)			
	0%	20%	40%	80%
Lean wet weight of the uterus	0,43±0,003[a]	0,13±0,012[a]	0,12±0,003[a]	0,09±0,003[b]

Note: $\bar{x}$=used to determine the mean value of a dataset; SD=used to assess the variability of the data.

Through proteolytic mechanisms, papain can damage the structural proteins in the uterine tissue, inhibiting the normal development of muscle cells and connective tissue that supports the uterus (Annaházi et al., 2021; Jaiswal et al., 2010). This damage may lead to uterine tissue atrophy, which is directly associated with a decrease in both the size and weight of the uterus, as reflected in the reduction in the lean wet weight of the uterus observed in the treated mice. Papain is also known to have the potential to cause changes in the hormonal balance within the body, which plays an essential role in the development and maintenance of uterine tissue. The observed reduction in uterine weight following treatment with papaya juice may also be related to hormonal disruptions caused by the consumption of papain. Papain can affect estrogen and progesterone levels, two primary hormones that regulate the reproductive cycle and the development of reproductive organs in mammals. A disturbance in this hormonal balance can lead to alterations in the structure and function of the uterus, ultimately resulting in a decrease in uterine weight. These findings align with the previous study, which showed that administration of papaya seed extract at a dose of 40 mg/kg body weight/day caused an increase in the volume of fluid in the uterus and the cessation of follicle formation at a dose of 160 mg/kg body weight/day due to hormonal disruption.

Conclusion

The administration of ripe papaya juice (Carica papaya) significantly reduced the uterine length and lean wet weight of mice. The observed decrease in uterine length following papaya juice treatment is likely attributed to the active compounds in papaya, especially the enzyme papain, which can affect cell proliferation and induce atrophy in uterine tissue. The implications of these findings focus on the impact of ripe papaya juice on reproductive health and its potential use in medical treatments or biomedical experiments. The effects on uterine length and weight may be linked to papains ability to influence hormonal balance and lipid metabolism, which could be considered in the use of papaya as a natural substance in treatments or research related to reproductive disorders. However, it is essential to note that these effects may depend on the dose and duration of administration, highlighting the need for further research on the safety and appropriate dosage before using papaya for therapeutic purposes.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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