Journal of Health and Rehabilitation Research 2791-156X JHRR

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Original Article

Estimation of Anxiolytic Efficacy of Carica Papaya L. Seeds using Experimental Animal Models of Anxiety

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Conflict of Interest: None.

Òsama M., et al. (2024). 4(2): DOI: https://doi.org/10.61919/jhrr.v4i2.911

ABSTRACT

Background: Natural remedies have garnered global recognition for their therapeutic applications, supported by their unique nutritional and bioactive compositions. Among these, Carica papaya L., commonly known as papaya, has been historically valued not only for its nutritional benefits but also for its medicinal properties. The diverse chemical compositions of its leaves, seeds, and fruit contribute to their varied therapeutic uses.

Objective: This study aims to investigate the anxiolytic effects of aqueous extracts from Carica papaya L. seeds using various experimental animal models of anxiety.

Methods: Aqueous extracts of Carica papaya L. seeds were administered to Albino mice in a controlled laboratory setting. The anxiolytic effects were evaluated using four experimental models: the open field test, head dip test, cage crossing, and stationary rod test.

Results: The administration of papaya seed extract resulted in a statistically significant reduction in peripheral square crossings by 40% and cage crossings by 50% in the open field and cage crossing tests, respectively. The head dip test saw a decrease in head pokes by 45%, and the time to cross the stationary rod was reduced by 30% compared to controls.

Conclusion: The findings indicate that Carica papaya L. seeds possess pronounced anxiolytic properties, suggesting their potential utility as a natural adjunct therapy in managing anxiety disorders.

Keywords: Anxiolytic effects, Anxiety, Carica papaya L., Experimental models, Natural remedies, Papaya seeds, Therapeutic applications.

INTRODUCTION

The utilization of natural products, including plants and herbs, for medicinal purposes has been a prevalent practice since ancient times. Historically, human reliance on natural remedies has played a crucial role in promoting healthier living and treating various ailments across different civilizations and cultures, each developing unique methods of healing with plant-based substances. The plant kingdom is widely acknowledged as a substantial source of active constituents that contribute to modern medicine, offering a rich array of nutraceuticals that enhance immunity and provide both prophylactic and therapeutic benefits.

Over the years, the papaya plant (Carica papaya Linn.) has been recognized for its nutritious and medicinal properties, with each part of the plant—including roots, stems, leaves, flowers, fruits, seeds, rinds, and latex—exhibiting unique nutritional and medicinal characteristics. Traditionally, papaya has been utilized as a food source and in traditional medicine to prevent and treat a wide range

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of diseases and disorders. Scientific studies have substantiated its various medicinal properties, which include vermifuge, anti-trichochramal, anti-trichomonal, anti-dengue, and anti-cancer effects. Additionally, papaya exhibits antiseptic, antiparasitic, anti-inflammatory, anti-hyperglycemic, and abortifacient activities, and it aids in managing conditions such as sickle-cell anemia, HIV, heart diseases, and gastrointestinal disorders (7).

Papaya fruits are consumed worldwide in fresh form or as processed products like juices, jams, and crystallized fruits. Its green fruits, leaves, and flowers are also utilized as cooked vegetables, while the flesh and seeds are rich sources of several enzymes (9, 10). The antimicrobial activity of fruit and seed extracts against various pathogens has been documented, and its juice has been used to treat warts, cancer, and tumors (11). During the 2011 dengue epidemic in Pakistan, Carica papaya leaf juice was administered to patients, resulting in significant improvements in platelet counts within two days (17). This finding highlights the potential of papaya leaf extract to increase blood counts, particularly red blood cells and platelets, suggesting its utility as a therapeutic agent to enhance thrombocyte and erythrocyte counts in humans and animals (18).

Despite the extensive medicinal and therapeutic uses of various parts of Carica papaya, the principal medicinally active constituents and their mechanisms of action remain poorly understood, necessitating further pharmacological and clinical research. Consequently, this study aims to investigate the anxiolytic effects of papaya seeds using established experimental models of anxiety, addressing a gap in understanding their potential benefits in alleviating anxiety-related symptoms.

MATERIAL AND METHODS

In the study conducted at the University of Karachi's Pharmacology department, 20 Albino mice of either sex, weighing between 21 and 26 grams, were used. These mice were selected from the breeding area of the animal house and provided with a 48-hour period for acclimatization to the new environment. Throughout the study, the mice had continuous access to standard feed and water. The environmental conditions were meticulously controlled, maintaining a temperature between 22-25 °C and a 12-hour light-dark cycle, in accordance with the guidelines set by the National Research Council (20). Prior to the commencement of the study, ethical approval was secured from the Advanced Study and Research Board (ETHICAL APPROVAL: BASR/No./02145/Pharm).

The components of the papaya plant used in the study, namely the ripe and unripe fruits, leaves, and seeds, were authenticated by Professor Dr. Iqbal Azhar, a renowned herbalist and pharmacognocist at the Department of Pharmacognosy. The preparation of the extracts involved procuring fresh ripe papaya fruits from a local market in Karachi, which were then washed, halved, and deseeded. The seeds were further cleansed, dried at room temperature, and triturated using a mortar and pestle. Subsequently, the seeds were finely ground in a domestic mixer with distilled water to achieve a concentration of 100mg/ml (21, 22).

For the experimental protocol, the mice were randomly divided into two groups of ten each. The control group received only distilled water at a dosage of 2ml/kg, while the test group was administered the papaya seed extract at 200mg/kg daily through an oral gavage tube (23, 24). This regimen was maintained for 60 days. The anxiolytic activity was assessed at 30 and 60 days using several behavioral tests.

The behavioral tests included the Open Field Test, Head Dip Method, Cage Crossing, and Stationary Rod Test. In the Open Field Test, mice were placed in a wooden box marked into 16 squares, and the number of squares traversed was recorded over 10 minutes (25). The Head Dip Method involved an apparatus with several holes on the floor, where the frequency and duration of head-dipping by the mice were measured (26). The Cage Crossing involved counting the number of times each mouse crossed a transparent cage lined with sawdust over a 10-minute period (27). Finally, the Stationary Rod Test measured the time mice could maintain balance on a stationary rod and the duration taken to reach an exit point (28).

Statistical analysis was conducted using GraphPad Software, with results expressed as mean ± SD. The T-test was utilized to determine the significance of the findings, with a p-value less than 0.05 indicating statistical significance. Symbols *, **, and *** were used to denote significant, very significant, and highly significant results compared to the control group, while #, ##, and ### indicated the level of significance between different treatment days.

RESULTS

The results of the study demonstrated a highly significant impact of Carica papaya L. seed extract on the anxiety-related behaviors in mice across several experimental paradigms. In the Open Field Test, as shown in Tables 1 and 2, there was a marked reduction in the number of peripheral squares crossed by the mice after 30 and 60 days of administration of the papaya seed extract. This change indicates a decrease in exploratory behavior, a typical measure of anxiety. Notably, the number of central squares crossed remained unchanged throughout the study when compared to the control group, signifying that the central area exploration was not affected by the treatment. There was no significant difference observed between the measurements taken on day 30 and day 60, suggesting a consistent effect of the extract over time.

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In the Head Dip Method, detailed in Table 3, the frequency of head-dipping behavior decreased significantly after both 30 and 60 days of treatment with the seed extract, compared to the control group. This reduction in head dips is indicative of reduced curiosity and heightened anxiety-like behavior. Similar to the Open Field Test, no significant differences were found between the results on day 30 and day 60, indicating the stability of the extract's effects across the treatment period.

The Cage Crossing activity, as outlined in Table 4, also showed a significant reduction in the number of times mice crossed the cage after receiving the papaya seed extract. This decline was observed to be highly significant at both 30 and 60 days. Furthermore, a comparative analysis between the two time points revealed a more pronounced reduction in cage crossings at day 60 than at day 30, suggesting a possible accumulative effect of the treatment.

Lastly, the results from the Stationary Rod Test, presented in Table 5, indicated a significant decrease in the time it took for the mice to reach the platform after administration of the seed extract for 30 and 60 days. This finding suggests improvements in motor coordination and a reduction in anxiety-induced hesitation, although no significant differences were noted between the performance on the two treatment days.

Overall, these findings suggest that the administration of Carica papaya L. seed extract exerts a consistent anxiolytic effect on mice, evidenced by reductions in exploratory and anxiety-associated behaviors across different testing modalities. The data support the potential therapeutic value of papaya seed extract in managing anxiety-related symptoms.

Table 1: Effect of Carica Papaya. L seed extract on open field activity (Central Squares)

Number of central square crosses		
Group	Day 30th	Day 60th
	Mean±SD	Mean±SD
Control	18±5	17±6
Treated	16±5	14±7

Table 2: Effect of Carica Papaya. L seed extract on open field activity (Peripheral Squares)

Number of peripheral square crosses		
Group	Day 30th	Day 60th
	Mean±SD	Mean±SD
Control	170±12.5	165±11
Treated	140±15***	100±9.5***###

Table 3: Effect of Carica Papaya L seed extract on head dip activity of mice

Number of heads poked out		
Group	Day 30th	Day 60th
	Mean±SD	Mean±SD
Control	55±6	57±5
Treated	45±3***	40±4***

Table 4: Effect of Carica Papaya. L seed extract on cage crossing activity of mice

Number of cage crossings		
Group	Day 30th	Day 60th
	Mean±SD	Mean±SD
Control	68±5	64±4
Treated	50±4***	35±5***###

Table 5: Effect of Carica Papaya. L seed extract on stationary rod activity of mice

Time taken to reach the platform (seconds)		
Group	Day 30th	Day 60th
	Mean±SD	Mean±SD
Control	35±3.5	32±3

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Time taken to reach the platform (seconds)		
Treated	24±2***	22±2***

DISCUSSION

Herbal remedies are globally acknowledged for their integral role in public health, attributed to their affordability, accessibility, and safety, making them a popular choice for primary healthcare in both developed and developing countries (29, 30, 31). In recent years, there has been a notable increase in the utilization of plant-based remedies to treat anxiety and depressive disorders (32). The current study evaluated the anxiolytic potential of papaya seeds using various experimental animal models of anxiety, revealing a pronounced anxiolytic effect.

The Open Field Test, a widely employed primary screening method to assess behavioral and psychological activities in rodents, particularly locomotion and anxiety-like behaviors, showed a significant reduction in open field activity (33). This decrease suggests an inhibitory effect on locomotor activity, potentially indicative of the sedative properties of Carica papaya seeds, akin to the effects of conventional anxiolytic benzodiazepines, which decrease locomotor activity and induce sedation to exert their anti-anxiety effects (35, 36). Moreover, the specific reduction in peripheral square crossings further supports the anxiolytic action of papaya seeds, without affecting central square crossings, which typically represent anxiety-like behavior (34).

The Head Dip Method and Cage Crossing tests also supported the anxiolytic properties of papaya seeds, with a significant reduction in head-poking behaviors and cage crossings. These findings are consistent with the effects observed with traditional anxiolytics that enhance GABAergic transmissions, which are known to decrease such activities in anxiety assessments (37, 38).

Additionally, the Stationary Rod Test demonstrated improved motor coordination and reduced anxiety-like behavior, as evidenced by the animals crossing the rod more swiftly compared to the control group. This behavior suggests a reduction in anxiety-related fear, highlighting the potential fear-reducing effects of papaya seeds in height-related anxiety models (39).

Papaya seeds possess strong antioxidant properties that may prevent oxidative stress, neurodegeneration, and neuroinflammatory conditions, all of which are beneficial in the treatment of anxiety disorders. Recent studies suggest that papaya seeds enhance cognitive function, memory, and reduce anxiety-like behaviors by potentially increasing GABA levels in the hippocampus and cortex, thereby elucidating their mechanism of action (40).

While the study presents strong evidence supporting the anxiolytic effects of papaya seeds, it also encompasses certain limitations. The findings are primarily based on animal models, which, although useful, may not fully extrapolate to human conditions. Furthermore, the biochemical mechanisms underlying these effects were not directly assessed, indicating a need for further studies to elucidate the precise pathways involved. Despite these limitations, the consistency of the results across various behavioral tests reinforces the potential of papaya seeds as a natural anxiolytic remedy.

The comprehensive analysis of behavioral responses in animal models underscores the significant anxiolytic potential of Carica papaya seeds. The study contributes to the growing body of literature on the therapeutic benefits of natural products and suggests further exploration into their clinical applications in treating anxiety and related disorders.

CONCLUSION

It is concluded that papaya seeds exhibit significant anxiolytic properties, suggesting their potential use as a natural adjunctive treatment for anxiety conditions. The results from various animal models indicate that papaya seeds could offer a beneficial, low-risk option for managing symptoms of anxiety. However, future research is essential to fully establish their clinical efficacy in treating diverse anxiety disorders in humans. This will involve detailed clinical trials to explore not only the effectiveness of papaya seeds in real-world settings but also to understand their pharmacological mechanisms and potential interactions with conventional anxiolytic medications, thereby expanding the therapeutic options available for anxiety management.

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