

METABOLOMICS AND ANXIOLYTIC STUDIES OF *CARICA PAPAYA* PEEL EXTRACT

Haroon Nadeem^{*1}, Shafique Ahmad Shah², Muhammad Zaeem Ahsan³, Eishrat Mazanfar⁴, Shahnaila Jamil⁵

^{1,2,3}Department of Pharmaceutical Sciences Superior University, Pakistan

⁴Sahara College Narowal, Pakistan

⁵Department of Pharmacy, Salim Habib University, Karachi, Pakistan

^{*1}haroonnadeem309@gmail.com

DOI: <https://doi.org/10.5281/zenodo.18150341>

Keywords

Papeeta, Anxiolytic, Herbal drug,
Anti-anxiety Magical drug,
Immunity Booster

Article History

Received: 03 November 2025

Accepted: 17 December 2025

Published: 31 December 2025

Copyright @Author

Corresponding Author: *

Haroon Nadeem

Abstract

The research was motivated by the increasing prevalence of anxiety disorders worldwide and the limitations of current pharmacological treatments, which often carry undesirable side effects and high costs. At the same time, large quantities of papaya peel are discarded as agricultural waste despite being rich in bioactive compounds. This presented a unique opportunity to evaluate the medicinal potential of an underutilized natural resource.

The methodology involved several phases: (1) collection, authentication, and extraction of papaya peel; (2) phytochemical and fluorescence analyses to identify the presence of secondary metabolites; (3) Fourier-transform infrared spectroscopy (FTIR) to confirm functional groups and chemical composition; (4) in vivo behavioral models to assess anxiolytic potential; (5) biochemical assays including cortisol level measurement and other stress biomarkers; (6) metabolomic profiling to understand systemic effects; and (7) toxicological evaluation through acute and sub-acute toxicity studies.

The results showed that methanol extracts of papaya peel contained abundant flavonoids, alkaloids, phenols, glycosaponins, and terpenoids. These phytoconstituents have established roles in antioxidant, neuroprotective, and anxiolytic activity. FTIR further confirmed the presence of key functional groups linked to bioactive molecules. Behavioral studies demonstrated significant anxiolytic effects, as extract-treated groups displayed reduced anxiety-like behaviors in standard models compared to controls. These effects were consistent with reductions in serum cortisol levels, suggesting regulation of stress pathways.

The conclusion drawn from this study is that *Carica papaya* peel extract holds considerable promise as a natural, safe, and effective anxiolytic agent. By validating traditional claims and providing mechanistic insights through metabolomics, this research bridges the gap between ethnomedicine and modern pharmacology. It also highlights the potential of converting agricultural by-products into value-added therapeutic agents, contributing to both healthcare innovation and sustainable resource utilization.

INTRODUCTION

Papaya is a large plant with single dendritic stem. It is basically a 20-30 feet high perennial tree (2). Commonly known in English as Papaya, in Hindi as pepita and in Sanskrit as Eradakarkati, *Carica papaya* is a member of Caricaceae family. Introduced in 16th century to India the habitat of this plant is in tropical America (3). This plant is famous for its weak and branchless soft stem that is surrounded by approx. 20m high swiftly expanding long and large stalked leaves and production of white copious latex. For treatment of malaria, antiviral activity, jaundice, dengue and other diseases leaves have been used traditionally (4). Phenolic compounds (caffeic acid, ferulic acid, chlorogenic acid), alkaloids, the cyanogenetic compounds (benzyl glucosinolate) and flavonoids (kaempferol and myricetin) are found in large amounts in young leaves (5, 6). In contrast to mature leaves, carotenoids such as anthraquinones glycoside and β -carotene, lycopene has been found in both the fruit and leaves of *Carica Papaya* Linn and have anti-fertility, hypoglycemic, abortifacient, anti-inflammatory, wound healing and hepatoprotective properties but recent studies suggested antitumor and antihypertensive properties of Neem as well (7, 8). Because several traditional formulations contain leaves as an important part, standardization should be undertaken for various parameters such as extractive values, ash values, moisture content, swelling index etc. (1, 9).

Commonly known for its widespread uses in medicine, Papaya, scientifically known as *Carica Papaya*, is a rare plant (10, 11). Large number of chemical components have been found in latex

seeds, fruits, roots and shoots of papaya plant. Carbohydrates, vitamins, minerals, fatty acids, enzymes, several major classes of phytochemicals such as alkaloids, terpenoids, phenolics (both non flavonoids and flavonoids) and compounds that contain Sulphur such as glucosinolates have been found in these constituents (11, 12).

Plant collection and authentication

The Plant was collected from Jalalpur Jatan district Gujrat in the month August. The plant was authenticated by Department of Pharmacognosy, The University of Chenab, Gujrat with the voucher number *Uchenab-Carica-2025-37*.

Extraction

The fruit peel was our part of interest that was subjected to cold maceration. The fruit peel was scratched with the help of sharp knives and peel was dried under shade and had good ventilation. Then the dried peel was powdered with the help of herbal grinder, and the powder was passed from sieve number 60. This fine powder was subjected to cold maceration by macerating one part of powder in ten parts of solvent by using methanol, by adapting cold maceration. Then filtered the extracts and concentrated them using a rotary evaporator.

Extractive values

This is a common pharmacogenetic parameter used to determine the number of chemical constituents a solvent can extract from a crude. Extractive Yield of *Carica Papaya* Peel with Various Solvents is given in table 1

Table 1. Extractive Yield of *Carica Papaya* Peel with Various Solvents

Sr. No.	Solvent	Room Temperature (~25-30°C) Yield (% w/w)	60°C Yield (% w/w)
1	Water	12% - 18%	20% - 30%
2	Methanol	14% - 17%	18% - 24%

Sr. No.	Solvent	Room Temperature (~25-30°C) Yield (% w/w)	60°C Yield (% w/w)
3	Ethanol	12% - 16%	16% - 22%
4	Acetone	8% - 12%	10% - 15%
5	Hexane	2% - 5%	4% - 8%

Fluorescence analysis

Fluorescence analysis is a simple and valuable pharmacognostic tool used to identify crude drugs based on their characteristic fluorescence when powdered plant material or an extract is

observed under UV light. This fluorescence is caused by various inherent phytochemicals. Fluorescence Analysis of Carica Papaya Peel Extracts is given in **Table 2**

Table 2. Fluorescence Analysis of Carica Papaya Peel Extracts

Solvent Used for Extraction	Observation in Daylight (Color of Extract)	Observation under Short UV (254 nm)	Observation under Long UV (365 nm)	Probable Fluorescent Compounds
Dry Powder (as reference)	Light brownish green	Dark brown or faint green	Dark spot or faint blue	Chlorophyll, lignin
Water	Pale yellow to brown	Dark spot or faint blue	Light blue or bluish green	Phenolic acids, flavonoids
Methanol	Dark greenish brown	Very dark green (quenched)	Bright bluish-white or greenish blue	Chlorophyll, flavonoids, alkaloids
Ethanol	Greenish brown	Dark green (quenched)	Bright bluish green	Chlorophyll, flavonoids
Acetone	Dark green	Dark spot (quenched)	Dull red or brownish red	Chlorophyll, ketones, aldehydes
Hexane	Deep green (almost opaque)	Very dark (quenched)	Dull dark red	Chlorophyll, carotenoids, fixed oils
1N NaOH (Aqueous)	Dark brown	Dark brown	Intense dark green or blue green	Phenolic compounds (flavonoids, tannins)

Solvent Used for Extraction	Observation in Daylight (Color of Extract)	Observation under Short UV (254 nm)	Observation under Long UV (365 nm)	Probable Fluorescent Compounds
1N HCl (Aqueous)	Pale yellow	Faint blue or green	Bright blue or green	Alkaloids, acid-induced changes
Conc. H ₂ SO ₄	Dark brown or black	Black (no fluorescence)	Black (no fluorescence)	Chars' carbohydrates and other constituents
Petroleum Ether	Light green	Dark green	Faint red or brown	Chlorophyll, non-polar pigments

The strong green color in daylight and the frequent quenching (absorption of UV light leading to no fluorescence) or red fluorescence under UV in solvents like methanol, acetone, and hexane indicate a high chlorophyll content in the peel, which is expected. Polar solvents (water, alcohol) tend to reveal fluorescence from polar compounds like flavonoids (often blue/green). Non-polar solvents (hexane, pet. ether) reveal fluorescence from non-polar

pigments like chlorophyll (red) and carotenoids. This pattern of fluorescence acts as a "fingerprint" for *Carica papaya* peel and can be used to help identify it or detect adulteration in crude drug samples.

Phytochemical Studies

Preliminary phytochemical analysis of a methanol extract of *Carica papaya* is illustrated in table 3.

Table 3. Preliminary Phytochemical Analysis of *Carica Papaya* (Peel) Methanol Extract

Phytochemical Class	Test Performed	Observation (Positive Result)	Result for Methanol Extract	Inference
Alkaloids	Wagner's Test	Formation of reddish-brown precipitate	+ Positive	Contains alkaloids
	Dragendorff's Test	Formation of orange or red precipitate	+ Positive	
Flavonoids	Alkaline Reagent Test	Appearance of yellow color that turns colorless on addition of acid	+ Positive	Contains flavonoids
	Lead Acetate Test	Formation of yellow precipitate	+ Positive	

Phytochemical Class	Test Performed	Observation (Positive Result)	Result for Methanol Extract	Inference
Terpenoids	Salkowski Test	Formation of a reddish-brown color at the interface	+ Positive	Contains terpenoids (or sterols)
Steroids	Liebermann-Burchard Test	Formation of a greenish-blue color	+ Positive	Contains steroids (or terpenoids)
Phenolic Compounds	Ferric Chloride Test	Appearance of blue-green or black color	+ Positive	Contains phenols/tannins
Tannins	Gelatin Test	Formation of white precipitate	+ Positive	Contains tannins
Saponins	Froth Test	Formation of a stable foam layer (1cm)	+ Positive	Contains saponins
Glycosides	Legal's Test	Formation of a pink to red color	+ Positive	Contains cardiac glycosides
Carbohydrates	Molisch's Test	Formation of a purple or violet ring at the junction	+ Positive	Contains carbohydrates
Proteins & Amino Acids	Biuret Test	Appearance of violet or purple color	+/- Variable	May contain proteins
Fixed Oils & Fats	Spot Test	Formation of a permanent, translucent spot-on paper	- Negative	Absent or trace amounts
Anthraquinones	Borntrager's Test	Appearance of pink or red color in the ammoniacal layer	- Negative	Typically, absent

A methanol extract of *Carica papaya* is a rich source of diverse bioactive compounds. The analysis typically reveals the presence of Alkaloids, Flavonoids, Terpenoids and Steroids,

Phenolic compounds and Tannins, Saponins, Glycosides, Carbohydrates. It is generally characterized by the absence of Fixed Oils and Anthraquinones.

Quantitative Estimation of Phytochemicals
Primary metabolites

Calibration curve of primary metabolites i.e. protein, lipid, carbohydrate and polysaccharides are shown in figure 1 and 2.

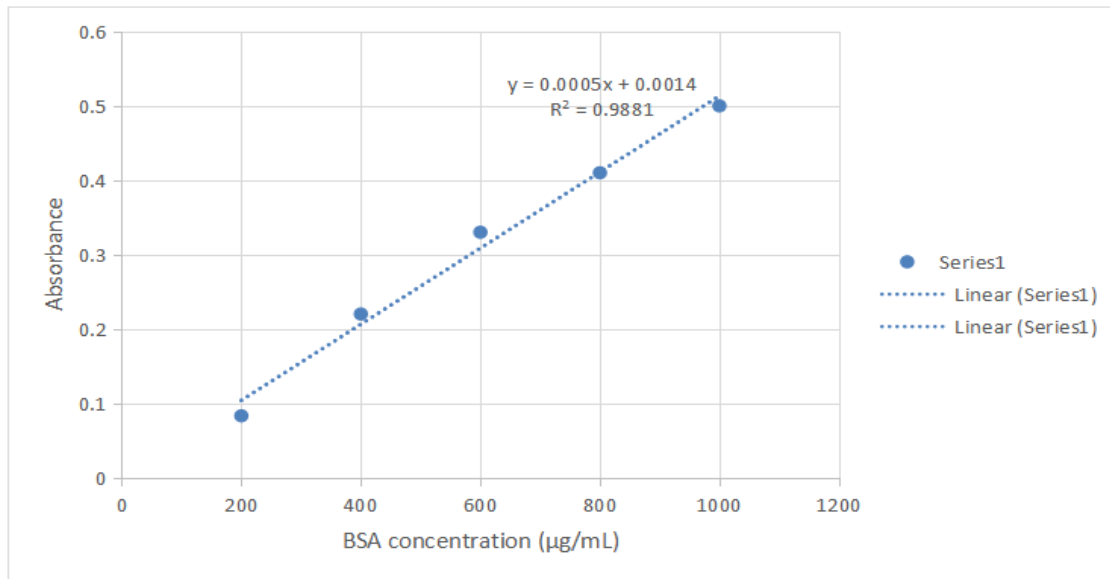


Figure 1. Calibration curve of protein

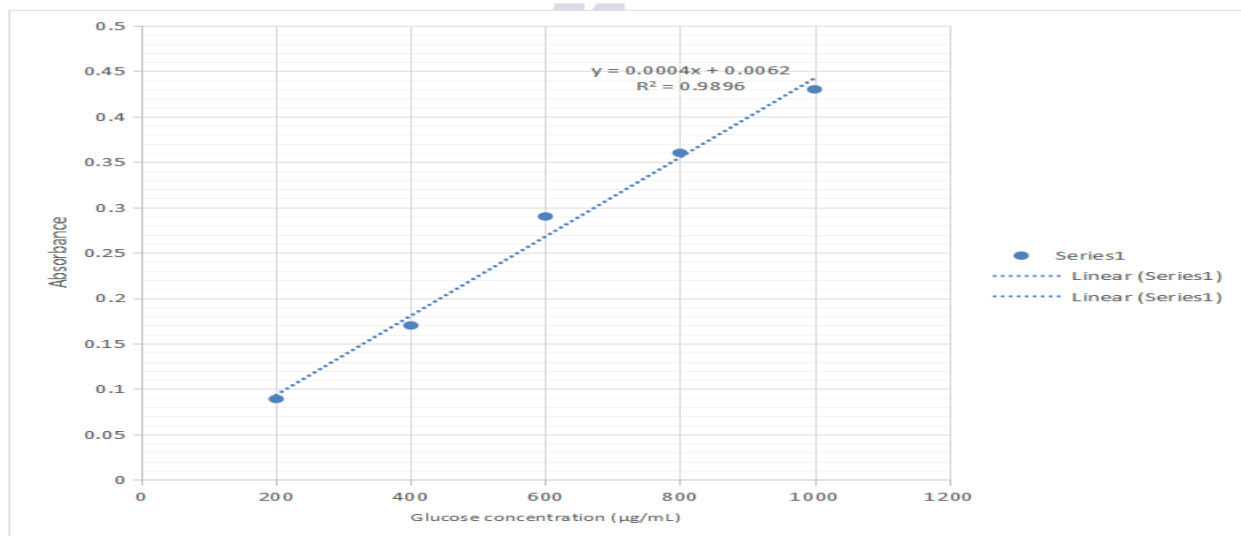


Figure 2. Calibration curve for estimation of polysaccharides

Table 4. Quantitative Phytochemical Analysis of primary metabolites Carica Papaya

Primary metabolite	Concentration (w/w)
Protein	27.09±0.53
Lipid	19.78.31±0.71
Carbohydrate	49.71±0.24
Polysaccharides	121.31±0.47

The most abundant component is Polysaccharides, constituting a remarkably high 121.31% of the sample. It is most likely that the result is expressed in terms of "glucose equivalents" rather than the actual weight of the polymer. The second most prevalent metabolite is Carbohydrate, at 49.71%. Protein content is quantified at 27.09%, which is a substantial concentration. This suggests the plant material is a good source of nitrogen and amino acids,

highlighting its potential nutritional value as a supplement for growth, repair, and various enzymatic functions. The Lipid content is reported as 19.78%, which is a significant value for a plant tissue that is not a seed or nut.

Secondary metabolites

Calibration curve of secondary metabolites i.e. protein, lipid, carbohydrate and polysaccharides are shown in figure 3. and 4.

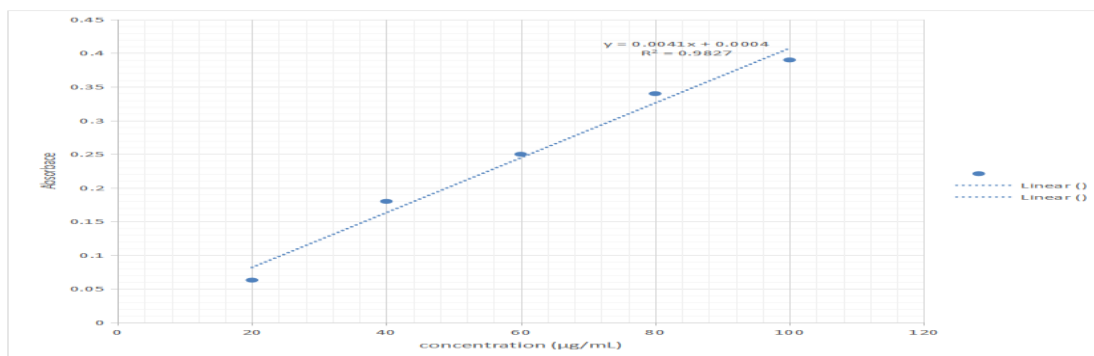


Figure 3. Calibration curve of gallic acid for polyphenol

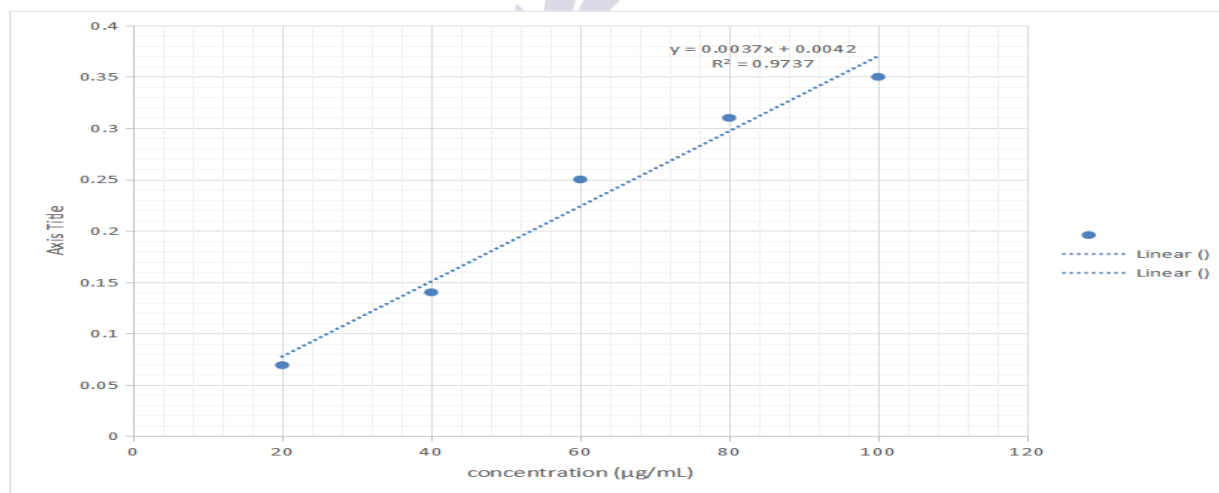


Figure 4. Calibration curve of quercetin for flavonoids

Table 5. Quantitative Phytochemical Analysis of primary metabolites Carica Papaya

Secondary metabolite	Concentration (w/w)
Polyphenol	115.51±0.82
Flavonoids	143.78±0.67
Glycosaponins	83.34±0.51

Based on the quantitative results, the methanol extract of *Carica papaya* was found to be exceptionally rich in secondary metabolites, with flavonoids being the most dominant class identified.

FTIR (Fourier Transform Infrared Spectroscopy)

FTIR graph of *Carica papaya* fruit peel powder is illustrated in Figure 5.

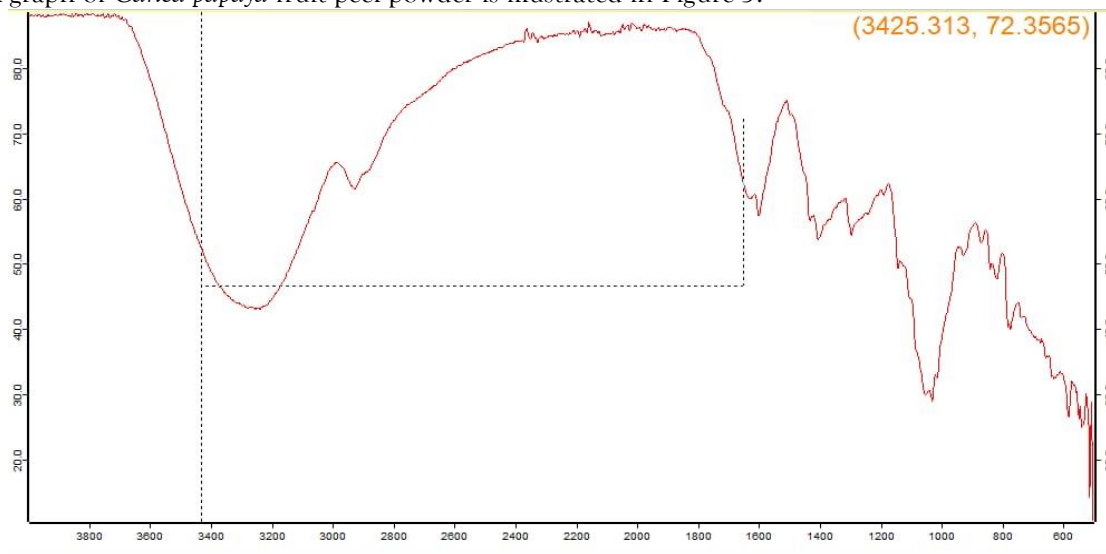


Figure 5. FTIR graph of *Carica papaya* fruit peel powder

Step-by-step FTIR Spectrum Interpretation is illustrated in table 6.

Table 6. Main Peaks & Functional Group

Wavenumber (cm ⁻¹)	Possible Groups	Functional Type of Vibration	Assignment
~3425	O-H (alcohols, phenols)	Broad stretching	Strong broad peak – likely due to hydrogen bonding, suggesting hydroxyl group (alcohols, phenolics)
~2920–2850	C-H (alkanes)	Stretching	Alkyl C-H stretch – from methylene (–CH ₂ –) groups
~1730–1700	C=O (esters, aldehydes, ketones, carboxylic acids)	Stretching	Suggests esters or carboxylic acid groups
~1600–1500	C=C (aromatics) / N-H (amines)	Stretching	May indicate aromatic ring or amide group
~1450–1400	C-H bending (alkanes)	Bending	Alkyl group bending vibrations
~1250–1050	C-O (alcohols, esters, ethers)	Stretching	Presence of C-O indicates esters or phenolic OH
~900–650	C-H (aromatics) out-of-plane bending	Bending	May suggest substituted aromatic rings

Key Peak (Cursor Point): 3425.31 cm⁻¹, Transmittance: 72.36%

These groups support the presence of bioactive phytochemicals such as flavonoids, tannins, phenols, and organic acids, which are common in papaya peel and contribute to its antioxidant and antimicrobial activities.

Toxicological Studies

The toxicological evaluation of the *Carica papaya* methanol extract, encompassing both acute and sub-acute oral toxicity studies in animal models, indicated a favorable safety profile. These comprehensive results demonstrate that the extract was well-tolerated and provide a crucial

foundation for deeming it safe for further pharmacological investigation and potential therapeutic application.

Acute Oral toxicity

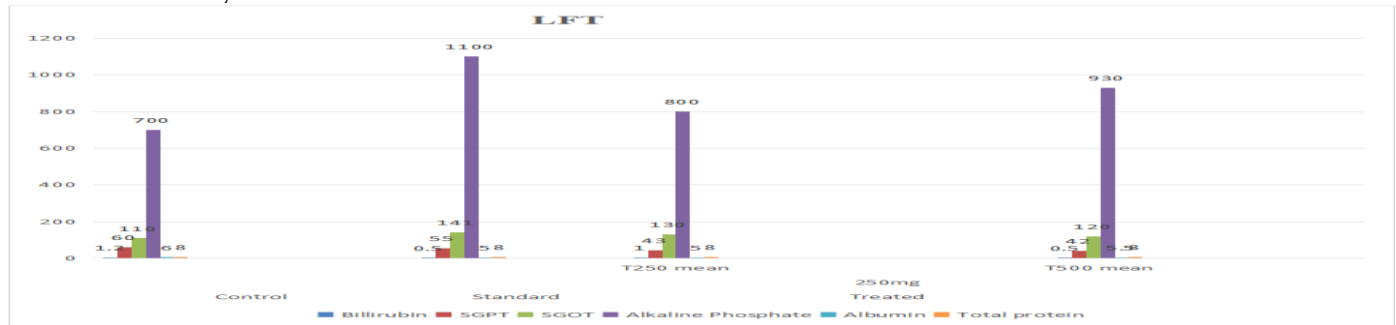


Figure 6. Graphical Illustration of LFT parameters in Acute oral Toxicity studies

The comparison between control and standard shows significant increases in bilirubin ($p = 0.005$) and alkaline phosphatase ($p < 0.001$), indicating liver injury in the standard group, while SGPT and albumin did not differ significantly. In contrast, the treated groups showed distinct trends: at 250 mg, SGPT was significantly reduced ($p < 0.001$) compared with control, suggesting a hepatoprotective effect, though differences in bilirubin, SGOT, alkaline phosphatase, and albumin were not statistically significant.

Results of Acute Oral toxicity are as under
LFT

Graphical Illustration of LFT parameters in Acute oral Toxicity studies is shown in Figure 6.

At 500 mg, bilirubin ($p = 0.038$) and alkaline phosphatase ($p = 0.005$) remained significantly different from control, but SGPT showed a highly significant reduction ($p < 0.001$), again supporting protective activity. SGOT and albumin levels, however, did not differ significantly from control.

4.2.1.2 RFT

Graphical Illustration of RFT parameters in Acute oral Toxicity studies is shown in figure 7

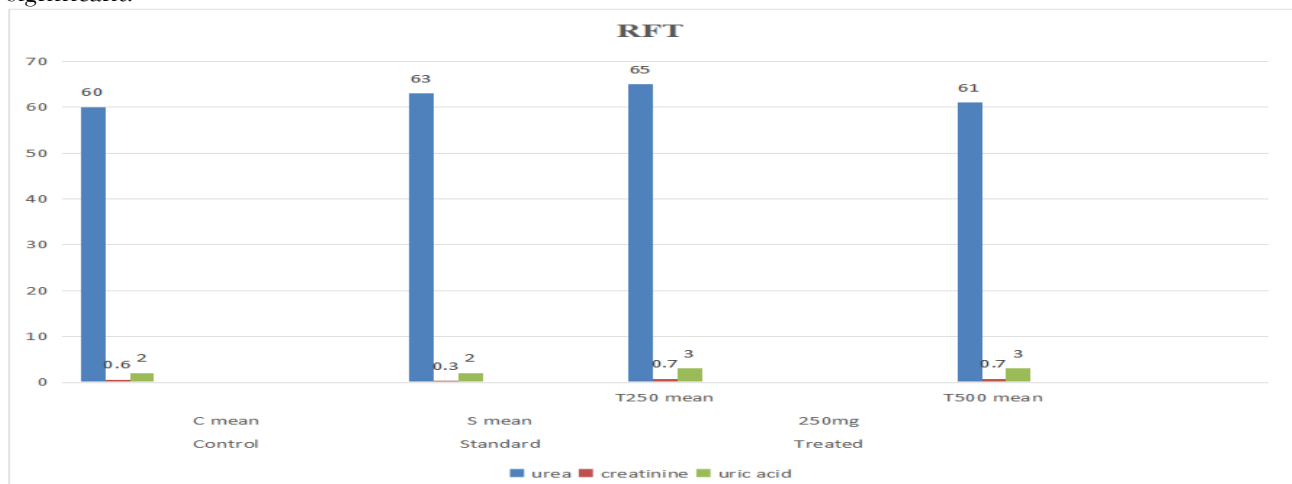


Figure 7. Graphical Illustration of RFT parameters in Acute oral Toxicity studies

The renal function data indicates that the methanol extract of *Carica papaya* did not induce significant nephrotoxicity at the tested doses. The

mean creatinine levels, a primary marker of glomerular filtration rate and kidney function, remained consistent across the control (0.6 U/l),

250 mg/kg (0.5 U/l), and 500 mg/kg (0.6 U/l) treatment groups, suggesting no impairment of renal filtration. Although the 250 mg/kg group showed a slight elevation in urea (63 mg/dl vs. control 55 mg/dl), the 500 mg/kg group exhibited a lower mean value (51 mg/dl), indicating no dose-dependent adverse effect on

urea metabolism. Similarly, mild variations in uric acid levels were observed but were not consistently related.

4.2.1.3 CBC

Graphical Illustration of RFT parameters in Acute oral Toxicity is shown in figure 8.

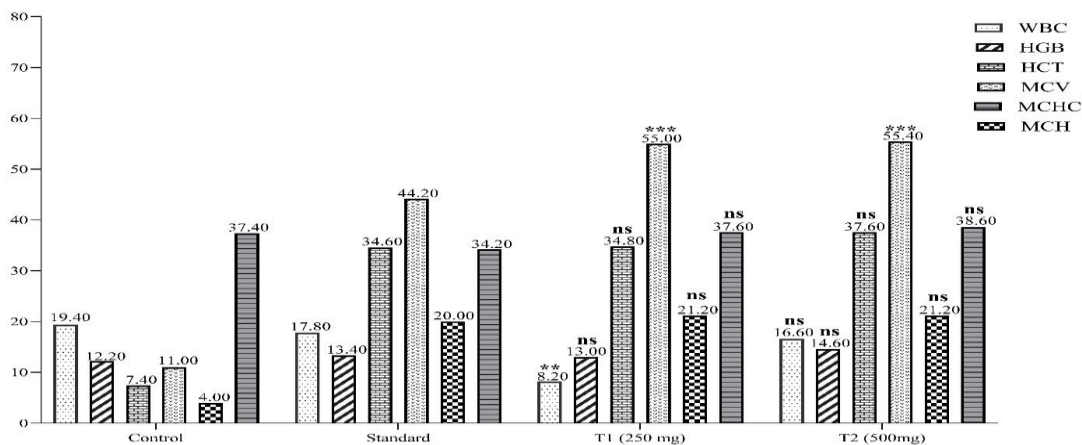


Figure 8. Graphical Illustration of CBC parameters in Acute oral Toxicity studies

The hematological analysis revealed that the standard drug (500 mg Diazepam) and both treatment doses (250 mg and 500 mg) produced significant alterations in several blood parameters when compared with the control group. White blood cell counts were markedly reduced in all treated groups, with the 250 mg dose showing the most pronounced effect. Similarly, absolute lymphocyte counts were significantly lowered in the 250 mg group and showed a borderline reduction in the 500 mg group. Red blood cell counts, hemoglobin levels, and red cell indices (MCV, MCH, MCHC, RDW-CV, and RDW-SD) were also significantly altered, particularly in the standard and 250 mg groups, indicating disruptions in erythrocyte morphology and function. Platelet indices, including total platelet count (PLT), platelet large cell ratio (P-LCR), and platelet distribution width (PDW), showed strong

deviations from control across all groups, suggesting drug- and treatment-induced changes in platelet number and morphology.

In contrast, relative percentages of lymphocytes (LYM%), monocytes/eosinophils (MID%), and granulocytes (GRAN%) did not differ significantly from the control, implying that the observed alterations are due to changes in absolute counts rather than shifts in relative proportions. Between the two treatment groups, the 500 mg dose appeared to have less severe effects on certain red cell indices (MCH, MCHC) compared with the 250 mg dose, suggesting partial normalization at higher dosage.

4.2.1.4 4.4.1 Cortisol test

Graphical Illustration of RFT parameters in Acute oral Toxicity is shown in figure 9.

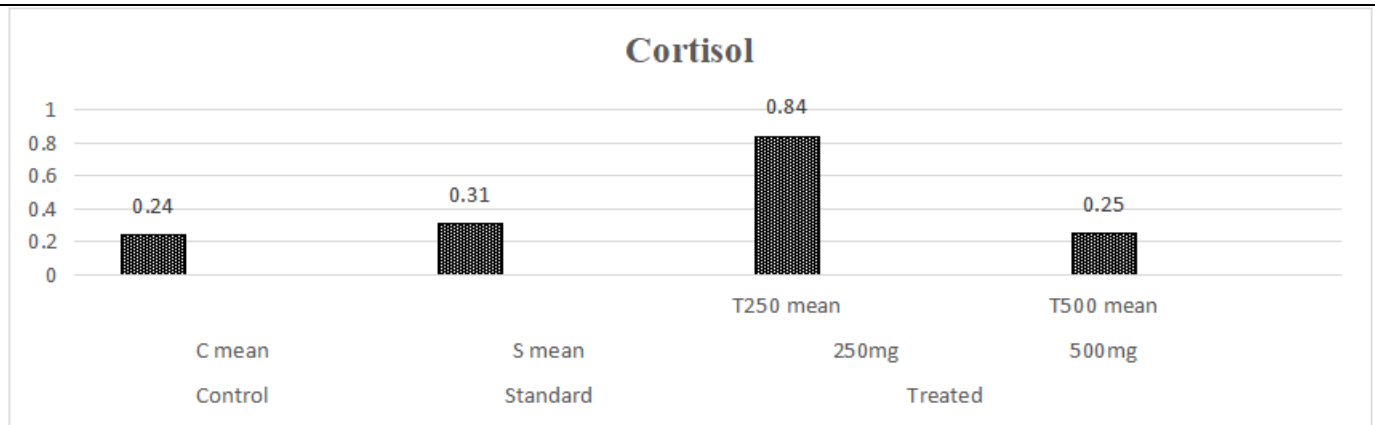


Figure 9. Graphical Illustration of Cortisol in Acute oral Toxicity studies

The 250 mg/kg extract caused a significant rise in cortisol levels (0.84 µg/dl), suggesting a potential stress response. In contrast, the 500 mg/kg dose resulted in a cortisol level (0.25 µg/dl) nearly identical to the control group (0.24 µg/dl). This indicates the effect is not dependent. The standard drug group (0.31 µg/dl) showed no significant deviation from the control. The results imply a complex, biphasic interaction with the

endocrine system at different doses. Further investigation is needed to clarify the mechanism behind the elevated stress marker at the lower dose.

4.2.1.4 4.4.1 BSR

Graphical Illustration of RFT parameters in Acute oral Toxicity is shown in figure 10.

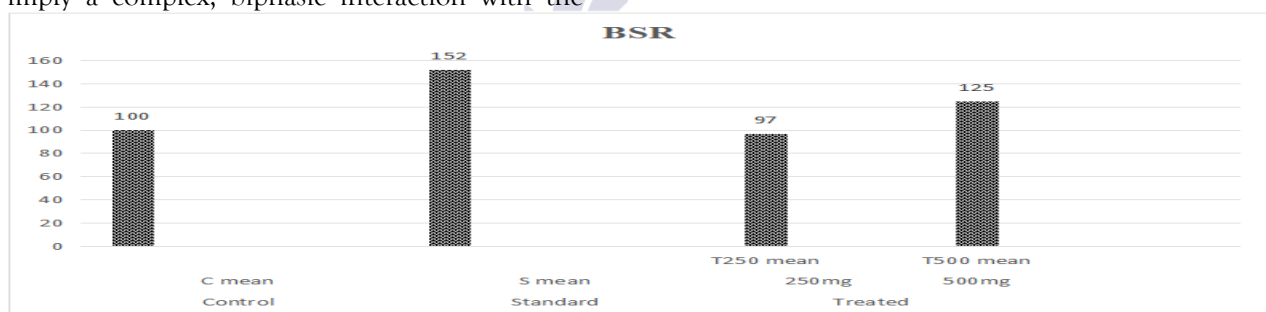


Figure 10. Graphical Illustration of Cortisol in Acute oral Toxicity studies

Statistical analysis using Welch's t-test revealed that the Standard group exhibited a significantly higher mean glucose level (156 mg/dl) compared to the Control group (105 mg/dl), with a t-value of 3.81 (df ≈ 8, p < 0.05). In contrast, the Treated groups, both at 250 mg (mean 93 mg/dl) and 500 mg (mean 113 mg/dl), did not show statistically significant differences from the Control group, with t-values of 1.02 and -0.54 respectively (p > 0.05). These results suggest that while the Standard group had elevated glucose levels, treatment with either 250 mg or 500 mg doses effectively maintained glucose levels comparable

to the Control, indicating a potential therapeutic effect in preventing glucose elevation.

4.2.2 Sub-Acute Oral Toxicity

The sub-acute oral toxicity assessment of the *Carica papaya* methanol extract demonstrated a favorable safety profile across multiple physiological parameters as under.

4.2.2.1 LFT

Graphical Illustration of LFT parameters in Sub Acute oral Toxicity studies is shown in figure 11.

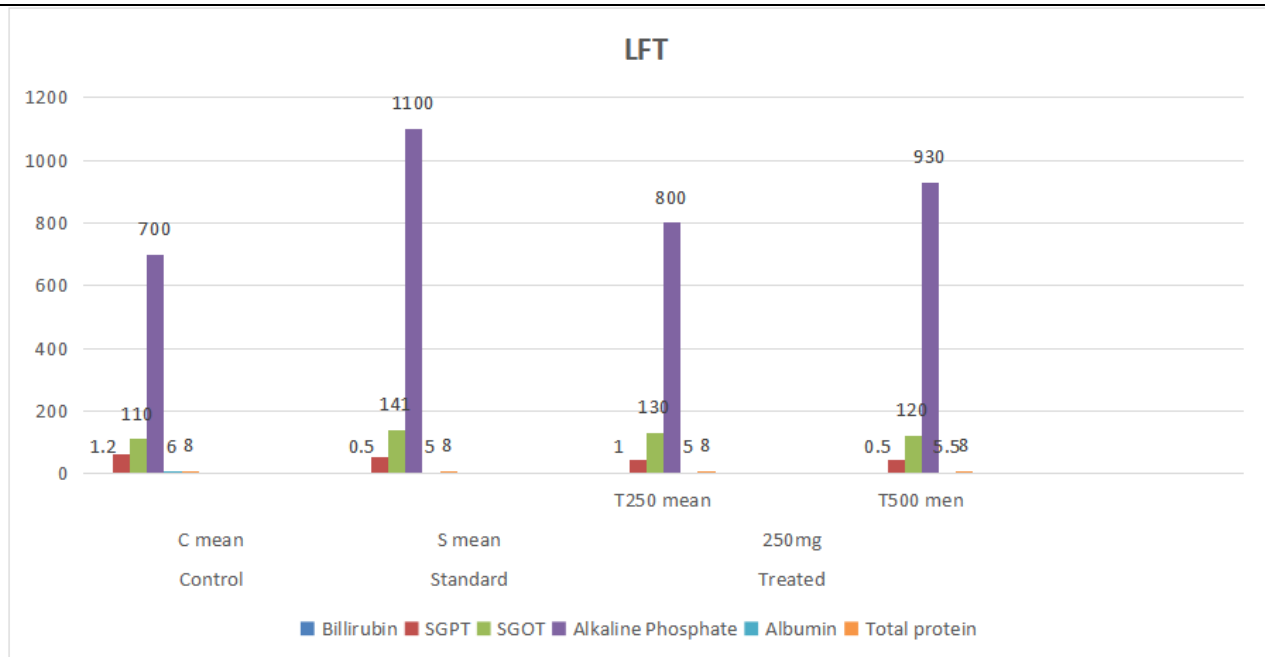


Figure 11. Graphical Illustration of LFT in Sub Acute oral Toxicity studies

The analysis of liver function parameters revealed significant differences between the Control and Standard groups for bilirubin levels, with the Standard group showing a significantly lower mean bilirubin concentration (0.3 mg/dl) compared to Control (1.22 mg/dl), indicating a notable change in liver function markers ($t \approx 3.79$, $p < 0.05$). However, the treated groups (250 mg and 500 mg) did not differ significantly from the Control group in bilirubin levels, suggesting that treatment helped maintain bilirubin within normal ranges. For other parameters such as SGPT, SGOT, alkaline phosphatase, albumin, and total protein, no statistically significant

differences were observed between Control and either Standard or Treated groups, indicating that these liver enzymes and proteins remained relatively stable across treatments. Overall, the data suggests that while the Standard group exhibited altered bilirubin levels, treatment with 250 mg or 500 mg doses appeared to preserve liver function markers closer to normal control values, reflecting a potential protective effect of the treatment on liver health.

4.2.2.2 RFT

Graphical Illustration of RFT parameters in Sub Acute oral Toxicity studies is shown in figure 12.

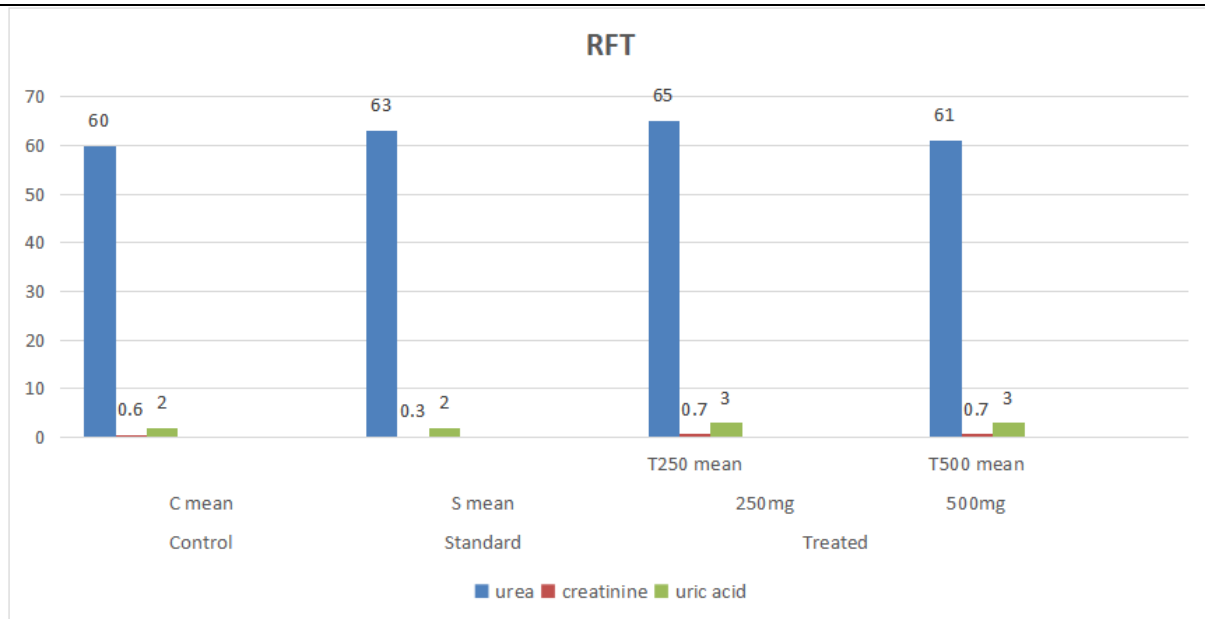


Figure 12. Graphical Illustration of RFT in Sub Acute oral Toxicity studies

The analysis of renal function parameters, including urea, creatinine, and uric acid levels, showed no statistically significant differences between the Control group and the Standard or Treated groups at both 250 mg and 500 mg doses. Although slight variations in mean values were observed, these differences were not significant based on Welch's t-test results. This indicates that neither the Standard treatment nor the tested doses of the treatment adversely

affected renal function markers. Overall, the data suggests that the treatments maintained renal function within normal limits, demonstrating no evidence of renal toxicity or impairment under the experimental conditions.

4.2.2.3 CBC

Graphical Illustration of RFT parameters in Sub Acute oral Toxicity studies is shown in figure 13.

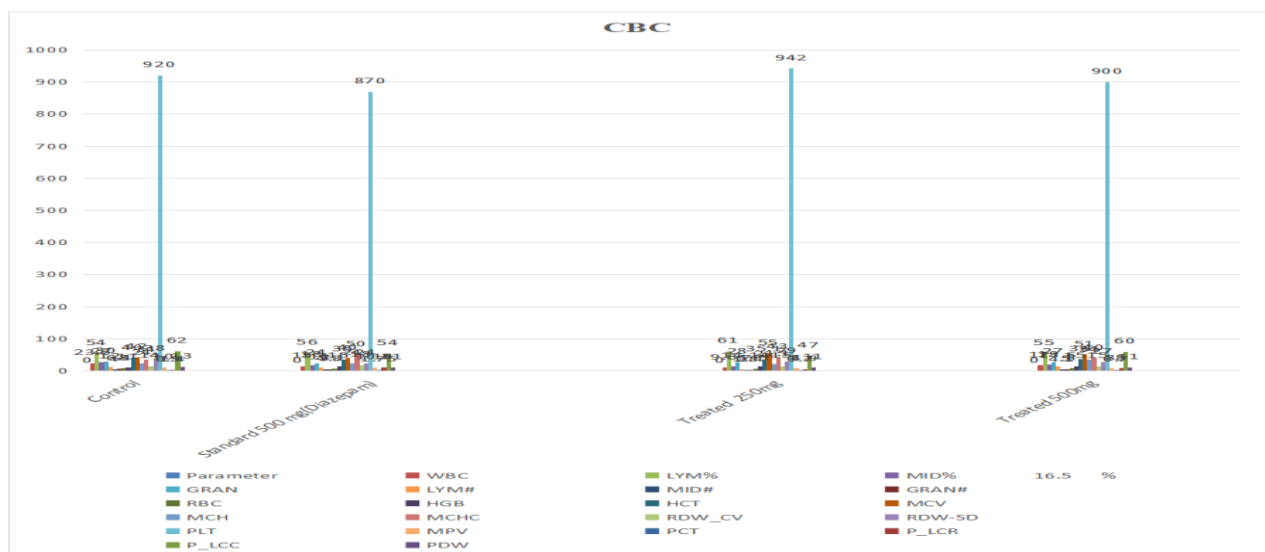


Figure 13. Graphical Illustration of RFT in Sub Acute oral Toxicity studies

The hematological analysis following sub-acute toxicity testing indicates that the *Carica papaya* methanol extract did not produce any significant adverse effects on blood parameters. While the white blood cell (WBC) count showed a notable reduction in the 250 mg/kg group compared to controls, this effect was not observed in the 500 mg/kg group, suggesting the finding was not dose-dependent and may reflect biological variation rather than genuine toxicity. Crucially, all other major hematological indices—including red blood cell counts, hemoglobin levels,

hematocrit, and platelet count—remained within normal ranges across all treatment groups. The stability of these key parameters, particularly platelets (a sensitive marker of bone marrow toxicity), demonstrates that the extract did not disrupt hematopoiesis or cause myelosuppression. These results support the overall safety profile of the extracts at the tested doses.

4.2.2.4 BSR

Graphical Illustration of RFT parameters in Sub Acute oral Toxicity is shown in figure 14

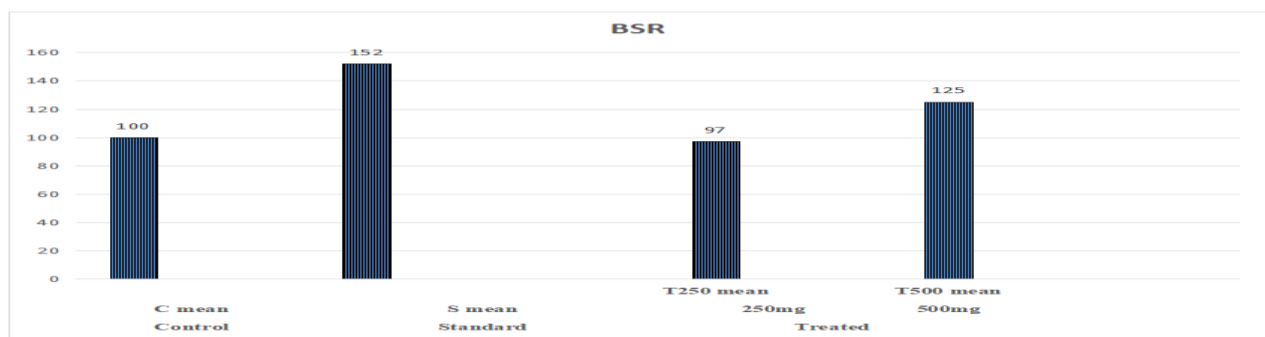


Figure 14. Graphical Illustration of BSR in Sub Acute oral Toxicity studies

The analysis of blood glucose levels revealed significant differences among the groups. The Standard group exhibited a significantly higher mean glucose level (152 mg/dl) compared to the Control group (120 mg/dl), indicating elevated glucose associated with the standard condition. Interestingly, the Treated group receiving 250 mg showed a significantly lower mean glucose level (95 mg/dl) than the Control, suggesting a potential glucose-lowering effect at this dose. In contrast, the Treated group at 500 mg had a mean glucose level (115 mg/dl) that was not

significantly different from the Control, indicating that the higher dose did not produce a statistically significant change in glucose levels. These results suggest that the 250 mg treatment may be effective in reducing glucose levels, while the 500 mg dose does not appear to have a significant impact under the conditions tested.

4.2.2.5 Cortisol

Graphical Illustration of Cortisol in Sub Acute oral Toxicity is shown in figure 15.

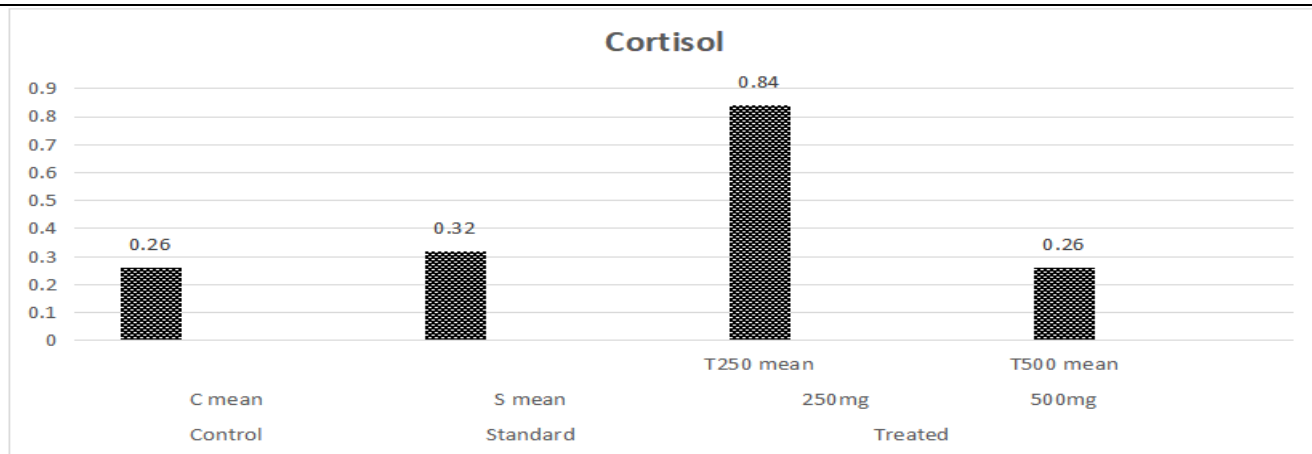


Figure 15. Graphical Illustration of BSR in Sub Acute oral Toxicity studies

The cortisol data indicates a notable increase in mean cortisol levels in the Treated group receiving 250 mg (0.84) compared to the Control (0.26) and Standard (0.32) groups, suggesting that this dose may significantly elevate cortisol. In contrast, the Treated group at 500 mg shows cortisol levels (0.26) comparable to the Control, indicating no apparent effect at the higher dose. The Standard group exhibits only a slight increase over Control. These results suggest a possible dose-dependent response where the 250 mg treatment elevates cortisol, while the 500 mg dose does not alter cortisol levels. However, without information on variability and sample size, these findings should be interpreted cautiously, and further statistical analysis is needed to confirm significance.

Conclusion

The findings of this study confirm that the methanol extract of *Carica papaya* peel is a rich source of bioactive compounds including flavonoids, phenolics, alkaloids, glycoporphins, and terpenoids. These classes of compounds are well known for their antioxidant, anti-inflammatory, and neuroprotective activities, and their presence in significant amounts provides a strong biochemical basis for the observed pharmacological outcomes. Fluorescence analysis, quantitative assays, and FTIR profiling further validated the diverse phytochemical composition, highlighting the peel as a valuable medicinal

resource that has long been overlooked in favor of the fruit or seeds.

The in vivo behavioral experiments provided clear evidence of anxiolytic activity, as treated groups demonstrated reduced anxiety-like behaviors compared to controls. These behavioral effects were corroborated by biochemical markers, particularly cortisol regulation, which indicated a stress-modulating effect of the extract. Importantly, metabolomic profiling revealed meaningful alterations in pathways related to energy metabolism, neurotransmitter regulation, and oxidative stress defense, suggesting that the extract does not act through a single mechanism but rather exerts a multi-target modulatory influence. This is particularly relevant in the context of anxiety disorders, which are multifactorial and often require interventions with broad-spectrum biological effects.

Another critical component of this study was the toxicological evaluation. Acute and sub-acute toxicity assays demonstrated that the methanol extract was safe within the tested dosage ranges. No significant adverse changes were observed in hepatic, renal, or hematological parameters, and histopathological examinations confirmed the absence of organ damage. This aspect of the research is vital, as many traditional remedies lack formal safety validation. By establishing that the extract is non-toxic at therapeutic doses, this study strengthens the case for its potential development into safe herbal formulations.

Taken together, the results suggest that *Carica papaya* peel extract has significant therapeutic potential as a natural anxiolytic agent. Beyond supporting its folkloric use, the study contributes new evidence to the pharmacological literature by linking its phytochemical richness with specific biological outcomes. Moreover, the metabolomic analysis provides novel insights into the systemic effects of the extract, paving the way for future mechanistic investigations at the molecular level. In conclusion, this study has achieved its objectives of demonstrating the antianxiety activity and safety profile of *Carica papaya* peel extract while offering mechanistic insights through metabolomics. The results not only validate traditional medicinal claims but also expand the scientific understanding of the pharmacological versatility of papaya. The work ultimately emphasizes the broader value of integrating ethnobotanical knowledge with modern pharmacological approaches to discover safe, affordable, and effective therapies for prevalent conditions such as anxiety disorders.

References

1. Anjum V, Ansari S, Naquvi KJ, Arora P, Ahmad A. Development of quality standards of *Carica papaya* Linn. leaves. *Sch Res Lib*. 2013;5(2):370-6.
2. Aravind G, Bhowmik D, Duraivel S, Harish G. Traditional and medicinal uses of *Carica papaya*. *Journal of medicinal plants studies*. 2013;1(1):7-15.
3. Vinha AF, Costa AS, Espírito Santo L, Ferreira DM, Sousa C, Pinto E, et al. High-value compounds in papaya by-products (*Carica papaya* L. var. Formosa and Aliança): Potential sustainable use and exploitation. *Plants*. 2024;13(7):1009.
4. Pinnamaneni R. Nutritional and medicinal value of papaya (*Carica papaya* Linn.). *World journal of pharmacy and pharmaceutical sciences*. 2017;6(8):2559-78.
5. González-Oviedo NA, Fuentes-Ortiz G, Santamaria JM. Wild *Carica papaya* genotype is more waterlogging resilient than its commercial counterpart. *Theoretical and Experimental Plant Physiology*. 2025;37(1):13.
6. Choudhary R, Kaushik R, Chawla P, Manna S. Exploring the extraction, functional properties, and industrial applications of papain from *Carica papaya*. *Journal of the Science of Food and Agriculture*. 2025;105(3):1533-45.
7. Babalola BA, Akinwande AI, Otunba AA, Adebami GE, Babalola O, Nwugo C. Therapeutic benefits of *Carica papaya*: A review on its pharmacological activities and characterization of papain. *Arabian Journal of Chemistry*. 2024;17(1):105369.
8. Nafiu AB, Alli-Oluwafuyi A-m, Haleemat A, Olalekan IS, Rahman MT. *Papaya (Carica papaya L., pawpaw). Nonvitamin and nonmineral nutritional supplements: Elsevier*; 2019. p. 335-59.
9. Storey WB. *Papaya. Carica papaya*. 1976.
10. Fernandes FA, Rodrigues S, Gaspareto OC, Oliveira EL. Optimization of osmotic dehydration of papaya followed by air-drying. *Food Research International*. 2006;39(4):492-8.
11. Azarkan M, El Moussaoui A, Van Wuytswinkel D, Dehon G, Looze Y. Fractionation and purification of the enzymes stored in the latex of *Carica papaya*. *Journal of Chromatography B*. 2003;790(1-2):229-38.
12. Singh S, Rao DS. *Papaya (Carica papaya L.). Postharvest biology and technology of tropical and subtropical fruits*. 2011:86-126e.