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**Immunomodulatory Activity of Methanolic Extract of *Carica papaya* Leaf on Biochemical Markers and Serum Level of IL-1 $\beta$  in Breast Cancer-Induced Wistar Rats**Alhassan, Hussaini Mohammed<sup>1</sup>, Sulaiman Aisha Galadima<sup>1</sup>, Abdullahi Isiyaku<sup>1</sup>, Usman Malami Aliyu<sup>2</sup>, Zafaru Muhammad Bunza<sup>3</sup>, Hamisu Abdullahi<sup>1</sup> and Mariya Bello<sup>1</sup>Department of Immunology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto-Nigeria <sup>1</sup>, Department of Radiotherapy and Oncology, Usmanu Danfodiyo University Teaching Hospital, Sokoto-Nigeria <sup>2</sup>, Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto-Nigeria <sup>3</sup>Author for Correspondence: halhassanmohd@gmail.com/ hussaini.alhasan@udusok.edu.ng/+234-803-970-5336. <https://dx.doi.org/10.4314/sokjmls.v9i2.27>**Abstract**

The most frequent malignant tumor in women worldwide is breast cancer. Up to 36% of oncological patients are breast cancer patients. In 2020, an estimated 2.089 million women received a breast cancer diagnosis and 685 000 deaths globally. The treatment options that are currently available for breast cancer are not only expensive but are associated with severe side effects. This study was designed to investigate the effect of *C. papaya* on biochemical and serum level of IL-1 $\beta$  in DAMB-induced breast cancer in Wistar Rats. A total of 35 rats were used in the study. The rats were divided into seven (7) groups of five (5) rats each. They were induced with 30mg/kg body weight of DMBA and observed for 14 days for signs of breast cancer development, and this was confirmed by presence of lump in the breast and also by measuring the size of the lump. The experimental groupings are: Group I: Normal Control, Group II: Positive Control (Induced with cancer but no treatment), III: Negative Control (Cancer +doxorubicin), Group IV, V and VI represent breast cancer rats treated with leaf extract of *Carica papaya* (300mg, 600mg and 1200mg respectively). Group VII represents cancerous rats, treated with 30mg of doxorubicin and 600mg of *Carica papaya*. This treatment was conducted in 14 days. At the end of the experiment, blood samples were collected for laboratory analysis, using standard techniques. The results indicated a statistically significant ( $p < 0.05$ ) decrease in serum level of IL-1 $\beta$  in all treated groups in a dose-dependent manner. Moreover, it was demonstrated that *C. papaya* does not increase liver enzymes and also have

protective effect on the kidneys. It could be concluded that *C. papaya* possesses immunomodulatory and anti-inflammatory properties and could be used in the management of breast cancer.

**Keywords:** *Carica papaya*, DMBA, IL-1 $\beta$ , Breast Cancer, Liver and Kidney

**Introduction**

Cancers involve the uncontrolled division cells of the body. Although cancer can develop in virtually any of the body's tissues, each type of cancer has its unique features, the basic processes that produce cancer are quite similar in all forms of the disease (Lyon *et al.*, 2020). Cancer begins when a cell breaks free from the normal restraints on cell division and begins to follow its own agenda for proliferation (Santana *et al.*, 2019). A tumor, or mass of cells, formed in these abnormal cells may remain within the tissue in which it originated (Nardin *et al.*, 2021). Breast cancer is a condition in which the breast's cells proliferate out of control. Like other malignancies, breast cancer occurs because of an interaction between the defective gene and the environment (Riggio, *et al.*, 2021). Signs and symptoms of breast cancer may include a breast lump or thickened area of skin that feels different from the surrounding tissue, a nipple that looks flattened or turns inward or changes in the color of the breast skin. In people with white skin, the breast skin may look pink or red (Khutaija *et al.*, 2017).

Breast cancer is the most frequent malignant tumor in women worldwide. Up to 36% of oncological patients are breast cancer patients. In 2018, an estimated 2.089 million women received

a breast cancer diagnosis (Nardin *et al.*, 2021). Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life. This trend is primarily attributable to the so-called Western lifestyle, which is known for its unhealthy eating habits, high levels of stress, and lack of exercise (Bellanger *et al.*, 2018). Despite the greater effectiveness of initial diagnosis or the rapid development of pharmacotherapy in recent years, breast cancer still remains the most cause of death in women in the world (Lyon *et al.*, 2020).

Interleukin-1 $\beta$  (IL-1 $\beta$ ), a potent pro-inflammatory cytokine, is essential for the host's defense mechanisms against infection and damage (Gloria and David, 2011). IL-1 $\beta$  is upregulated in breast neoplasm initiation and development. Variations in IL-1R and IL-1 $\beta$  have also been linked to the development of breast tumors (Muhammad *et al.*, 2021).

Natural plant products have shown promising results as anti-tumor and anti-cancer agents, one of which is *Carica papaya*. Because of its abundance of vitamins—including vitamins A, B, and C—monounsaturated fatty acids, proteins, carbohydrates, high levels of alkaloids like carpaine and pseudocarpaine, and other bioactive substances, papaya is regarded as a miraculous fruit (Koun *et al.*, 2022). Papaya has been associated with chemo-preventive qualities, which include activating tumor-suppressor genes, transcriptionally deactivating oncogene products, and reducing oxidative damage by scavenging free radicals (Anilkumar *et al.*, 2020). The papaya seed and pulp contain benzyl glucosinolate, which is converted by the enzyme myrosinase to benzyl isothiocyanate, which has anticancer effects and has been shown to inhibit the growth of certain cancer cell lines (Pathak *et al.*, 2014). Secondly, papaya fruits are abundant in lycopene, which has been shown to be effective in preventing the development of certain cancers, including prostate cancer (Rahmat *et al.*, 2019). Second, lycopene, which is abundant in papaya fruits, has been proven to be efficient in destroying liver cancer cells (Alotaibi *et al.*, 2019). When used on acute promyelocytic leukaemia, papaya seed extract had anticancer properties. Furthermore, the

ability of papaya seeds to successfully stop the development of prostate cancer cells suggests that they may have anticancer effects (Wormann, 2019). Papaya leaves also have an anticancer effect in addition to decreasing the responses of solid cancer cells in the cervix, breast, liver, lung, and pancreas (Bonofiglio *et al.*, 2016).

According to previous studies, liver injury from herbal preparation ranges from mild elevation of liver enzymes to severe hepatic failure requiring liver transplant, as such knowledge about the potential adverse effect of papaya should be increased (Pak *et al.*, 2014). However, limited data exists with respect to its potential toxic effects. Hence, this research is designed to study the Immunomodulatory activity of methanolic leaf extract of *Carica papaya* on biochemical parameters and expression of IL-1 $\beta$  in breast cancer-induced Wistar rats.

## Materials and Methods

### Study Area

The study was conducted in the Department of Immunology, School of Medical Laboratory Sciences and Animal house of the Faculty of Pharmaceutical Sciences, Usmanu Dandodiyo University, Sokoto (UDUS).

### Plant Collection and Authentication

The fresh leaves of *Carica papaya* were collected from Runjin Sambo area, Sokoto state, Nigeria and were identified and authenticated by Herbarium Officer at Herbarium, Faculty of Pharmaceutical Science, UDUS and a voucher number: PGS/UDUS/CA/0006 was assigned

### Preparation of Plant Extract

The leaves were washed under running water to remove surface pollutants then allowed to shade dried and grinded into a coarse powder and the powder was weighed using weighing balance and kept in an airtight container. Five hundred grams (500g) of the obtained powder was soaked in 1.5 litres of methanol. The mixture was vigorously mixed and allowed to stand at room temperature for 72 hours to ensure maximum extraction. The liquid extract obtained was filtered using Whatmann no.1 filter paper. The filtrate was placed in a water bath pre-set at a temperature of 50°C and allowed to evaporate. The obtained

residue was then transferred into a transparent wide mouthed screw-caped air-tight universal sterile container which was labelled and stored in a refrigerator at 40°C Until required for use.

### Ethical approval

The study was approved by the Animal ethics committee of Usmanu Danfodiyo University Sokoto. The experiments were conducted in accordance with the institutional Animal Ethics Committee guidelines as well as international accepted practices for use and care of laboratory animals as contained in United States guidelines.

### Phytochemical Screening

Phytochemical screening of methanolic leaf extracts of *Carica papaya* was conducted to ascertain chemical metabolites that are naturally produced by *Carica papaya* leaves using qualitative method.

### Experimental Animals

A total of forty-seven (47) females Albino rats (aged 8-12weeks old), weighing between 100-160g were purchased from the Animal Research

Centre (ARC) of the Ahmadu Bello University (ABU), Zaria, Nigeria. The rats were kept in the Animal House Department of Pharmacology, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto (UDUS), Nigeria. With alternating 12 hours of light and 12 dark cycles at ambient temperature. Food and water were available to the rats' ad libitum, and they were allowed to acclimatize to standard laboratory condition for 7 days. All the experimental protocols were in compliance with the Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines.

### Experimental Design

The total thirty-five (35) albino rats were randomly divided into seven groups (7), comprising of five (5) rats per group. All the groups were fed with basal diet and clean water. The test groups were induced with DMBA 30mg/kg body weight as described by (Alhassan *et al.*, 2021). Table 1 shows the Experimental Design

**Table 1: The Experimental Design**

S/N	Group (n= 5)	Treatment Options
1	Group I	Normal Control rats received only standard feed and clean water
2	Group II	Albino rats induced with breast cancer (DMBA) only received standard feed and clean water
3	Group III	DMBA induced breast cancer albino rats were treated with 10mg/kg body weight of Doxorubicin only and received standard feed and clean water
4	Group IV	DMBA induced breast cancer albino rats were treated with 300mg/kg body weight of methanolic extract <i>C. papaya</i> .
5	Group V	DMBA induced breast cancer albino rats were treated with 600mg/kg body weight of methanolic extract <i>C. papaya</i> .
6	Group VI	DMBA induced breast cancer albino rats were treated with 1200mg/kg body weight of methanolic extract <i>C. papaya</i> .
7	Group VII	DMBA induced breast cancer albino rats were treated with 600mg/kg body weight of methanolic extract <i>C. papaya</i> and 5mg/kg body weight of Doxorubicin

### Induction of Mammary Tumour

A single dose of 30mg/kg body weight of 7,12-dimethyl benzene anthracene (DMBA) was administered to the albino rats, subcutaneously and the rats were observed for development of breast cancer after 14 days of induction (Alhassan *et al.*, 2021).

### Sample Collection and Processing

After 4 weeks the rats were anaesthetized in glass jar containing weight wool soaked with chloroform. The blood sample was collected from the rats through cardiac Puncture into a clean, plain and EDTA (ethylene diamine tetra acetic acid) container and breasts of the rats were then removed and placed inside a container filled with 10% formol saline. The rats were later sacrificed through lumber dislocation. The sample collected in the plain tubes were centrifuged at 4000 rpm for 10 minutes, the obtained serum was then transferred into labelled sterile cryovials using Pasteur pipette and stored in a freezer at  $-20^{\circ}\text{C}$  until the time of assay.

### Determination of Serum IL-1 $\beta$ Concentration

The serum level of IL-1 $\beta$  was determined using quantitative sandwich ELISA techniques (Beijing Solarbio Science and Technology Science and Technology Co., Ltd, China). The procedure was followed according to manufacturers' instructions. The minimum detectable dose was 15 pg/mL.

### Evaluations of Liver Biomarkers

The liver biomarkers evaluated included Alanine amino transferase (ALT) (Colorimetric method), serum aspartate amino transaminase (AST) (Colorimetric method), serum alkaline phosphatase (ALP) (Colorimetric endpoint), Total protein estimation (Biuret reaction), Albumin estimation (Bromocresol green method), total and direct bilirubin.

### Evaluations of Kidney Biomarkers

The kidney biomarkers evaluated included serum creatinine concentration (Jaffe's method), Urea Estimation (Diacetylmonoxime method), and Electrolyte estimation (Ion Selective Electrode (ISE) method using semi-automated auto-analyzer) (Kavanagh and Mills, 1997).

### Statistical Analysis

The results obtained were transferred into SPSS version 27 for analysis. Continuous variables were expressed as mean and standard deviation (SD). Analyses of variance (ANOVA) were performed to explore differences on variables across the groups. Bonferroni test was used as post hoc test to compare some groups. The p value 0.05 was used to decide the level of statistical significance.

### Result Presentations

#### Qualitative Phytochemical Screening of *Carica Papaya* Leaf Extract

The qualitative phytochemical constituent in the leaf of *c. papaya* was determined by testing for the presence of the following constituent's carbohydrate, saponin, tannin, phenol, flavonoid, protein, amino acid, cardiac glycosides, steroids and alkaloid.

#### Effect of Methanolic Leaf Extract of *Carica papaya* on Liver Biomarkers in DAMB Induced Breast Cancer in Wistar Rats

The effect of methanolic leaf extract of *Carica papaya* on liver function parameters (AST(UI/L), ALP(UI/L), ALP(UI/L), TP(g/L), ALB(g/L), DB (mg/dL) and TB (mg/dL) in DAMB- induced breast cancer in Wistar rat is shown in table 3 The aspartate amino transaminase (ALT) in the normal control group ( $7.00\pm 1.15$ ) was significantly lower than those in the positive control group ( $10.00\pm 0.57$ ), negative control group ( $10.33\pm 0.33$ ), cancer + 300mg group ( $9.33\pm 1.20$ ), cancer + 600mg group ( $12.00\pm 0.57$ ), cancer + 1200mg group of extract ( $13.67\pm 0.66$ ) and cancer + 600mg+0.3mg of doxorubicin ( $9.00\pm 0.00$ ) ( $p<0.05$ ). The mean values in the cancer + 600mg of extract group, however, was significantly higher than those in the normal control group, cancer + 300mg group of extract and cancer + 600mg+0.3mg of doxorubicin group ( $p<0.5$ ). The mean value in the cancer + 1200mg of extract, was significantly higher than those in the normal control group, positive control group, negative control group, cancer+300mg group, cancer+1200mg group of extract and cancer + 600mg+0.3mg of doxorubicin group ( $p<0.5$ ). The alanine aminotransferase (ALT) in the normal control group ( $8.00\pm 0.57$ ) were significantly lower than those in the negative control group ( $11.33\pm 0.33$ ), cancer + 1200mg of extract group ( $13.00\pm 1.52$ ) and cancer + 600mg+0.3mg of doxorubicin group ( $12.33\pm 0.66$ )

( $p < 0.5$ ), the mean value in the positive control group ( $9.33 \pm 1.45$ ), was significantly lower than those in the cancer + 1200mg ( $13.00 \pm 0.33$ ) and cancer + 600mg group of extract + 0.3mg of doxorubicin group ( $12.33 \pm 0.66$ ) ( $p < 0.5$ ), the mean values in the cancer + 1200mg of extract group ( $13.00 \pm 0.33$ ) were significantly higher than those in the normal control group ( $8.00 \pm 0.57$ ), positive control group ( $9.33 \pm 1.45$ ), cancer + 300mg group ( $10.00 \pm 1.00$ ), cancer + 600mg group of extract group ( $10.33 \pm 0.33$ ) ( $p < 0.5$ ). The mean alkaline phosphatase (ALP) value of the normal control group ( $89.33 \pm 0.88$ ), positive control group ( $88.00 \pm 2.30$ ), negative control group ( $90.00 \pm 1.00$ ), cancer + 300mg group ( $88.67 \pm 3.48$ ), cancer + 600mg group ( $93.00 \pm 1.00$ ), cancer + 1200mg group of extract ( $90.00 \pm 1.52$ ) and cancer + 600mg+0.3mg of doxorubicin group ( $89.67 \pm 0.66$ ) were not significantly different ( $p > 0.05$ ). The total protein (PT) in the normal control group ( $68.33 \pm 0.33$ ) and positive control group ( $68.67 \pm 0.03$ ) were significantly higher than those in the negative control group ( $65.00 \pm 0.57$ ), cancer + 300mg group ( $64.67 \pm 0.33$ ), cancer + 600mg group ( $64.33 \pm 1.45$ ), cancer + 1200mg of extract group ( $64.00 \pm 0.57$ ) and cancer + 600mg+0.3mg of doxorubicin group ( $64.00 \pm 1.15$ ) ( $p < 0.05$ ). The mean serum albumin (ALB) value of the normal control group ( $38.33 \pm 0.33$ ), positive control group ( $38.33 \pm 0.88$ ), negative control group ( $37.00 \pm 0.57$ ), cancer + 300mg group ( $38.33 \pm 0.88$ ), cancer + 600mg group ( $37.67 \pm 0.33$ ), cancer + 1200mg group of extract ( $37.67 \pm 0.67$ ) and cancer + 600mg+0.3mg of doxorubicin group ( $36.00 \pm 1.73$ ) were not significantly different ( $p > 0.05$ ).

The mean direct bilirubin (DB) value of the normal control group ( $0.20 \pm 0.00$ ), positive control group ( $0.20 \pm 0.00$ ), negative control group ( $0.23 \pm 0.03$ ), cancer + 300mg group ( $0.20 \pm 0.00$ ), cancer + 600mg group ( $0.26 \pm 0.03$ ), cancer + 1200mg group of extract ( $0.26 \pm 0.03$ ) and cancer + 600mg+0.3mg of doxorubicin group ( $12.46 \pm 12.26$ ) were not significantly different ( $p > 0.05$ ). The mean total bilirubin (TB) value of the normal control group ( $0.73 \pm 0.03$ ), positive control group ( $0.76 \pm 0.06$ ), negative control group ( $0.80 \pm 0.57$ ), cancer + 300mg group ( $0.73 \pm 0.08$ ), cancer + 600mg group ( $0.76 \pm 0.03$ ), cancer + 1200mg group of extract ( $0.90 \pm 0.05$ ) and cancer + 600mg+0.3mg of doxorubicin group ( $0.83 \pm 0.03$ ) were not significantly different ( $p > 0.05$ ).

### **Effect of Methanolic Leaf Extract of *Carica papaya* on Kidney Biomarkers in Breast Cancer-induced Wistar Rats**

The effect of methanolic leaf extract of *Carica papaya* on kidney function parameters (urea (mmol/L), creatinine (mmol/L),  $\text{Na}^+$ (mmol/L),  $\text{K}^+$ (mmol/L),  $\text{HCO}_3^-$ (mmol/L) and  $\text{Cl}^-$ (mmol/L), in DAMB induced breast cancer in Wistar rat is shown in table 4. The serum urea level in the negative control group ( $7.56 \pm 0.88$ ) was significantly higher than those in the normal control group ( $4.83 \pm 0.12$ ), positive control group ( $5.93 \pm 0.14$ ), cancer + 300mg group ( $4.93 \pm 0.46$ ), cancer + 600mg group ( $5.49 \pm 0.51$ ), cancer + 1200mg group of extract ( $4.90 \pm 0.70$ ) and cancer + 600mg+0.3mg of doxorubicin ( $6.26 \pm 0.20$ ) ( $p < 0.05$ ). The mean values in the cancer + 600mg+0.3mg of doxorubicin, however, was also significantly higher than normal control group, cancer + 300mg group and cancer + 1200mg group of extract ( $p < 0.05$ ). The serum creatinine level in all the groups i.e. the normal control group ( $0.76 \pm 0.03$ ), positive control group ( $0.86 \pm 0.03$ ), negative control group ( $0.83 \pm 0.03$ ), cancer + 300mg group ( $0.80 \pm 0.05$ ), cancer + 600mg group ( $0.83 \pm 0.08$ ), cancer + 1200mg group of extract ( $0.90 \pm 0.00$ ) and cancer + 600mg+0.3mg of doxorubicin ( $0.83 \pm 0.03$ ) were not significantly different ( $p > 0.05$ ). The mean potassium (K) value of the normal control group ( $4.33 \pm 0.13$ ), positive control group ( $4.73 \pm 0.33$ ), negative control group ( $4.70 \pm 0.05$ ), cancer + 300mg group ( $4.30 \pm 0.11$ ), cancer + 600mg group ( $4.36 \pm 0.23$ ), cancer + 1200mg group of extract ( $4.50 \pm 0.10$ ) and cancer + 600mg+0.3mg of doxorubicin group ( $4.70 \pm 0.10$ ) were not significantly different ( $p > 0.05$ ). The serum sodium (Na) level in the normal control group ( $145.00 \pm 0.577$ ) was significantly higher than those in the positive control group ( $141.00 \pm 1.00$ ), negative control group ( $138 \pm 0.88$ ), cancer + 600mg, group ( $139.00 \pm 0.57$ ), cancer + 1200mg group of extract ( $140.67 \pm 1.20$ ) and cancer + 600mg+0.3mg of doxorubicin ( $140.33 \pm 2.08$ ) ( $p < 0.05$ ). The mean values in the cancer + 300mg of extract group, however, was significantly higher than negative control group ( $138 \pm 0.88$ ), and 600mg of extract group ( $139.00 \pm 0.57$ ) ( $p < 0.05$ ). The mean chloride ion ( $\text{Cl}^-$ ) value of the normal control group ( $101.33 \pm 1.85$ ), positive control group ( $99.00 \pm 0.57$ ), negative control group ( $98.67 \pm 0.33$ ), cancer + 300mg group ( $102.67 \pm 2.33$ ), cancer + 600mg group ( $99.67 \pm 0.88$ ), cancer + 1200mg group of extract ( $99.67 \pm 0.66$ ) and cancer + 600mg+0.3mg of doxorubicin group

(101.00±1.15) were not significantly different ( $p>0.05$ ). The serum bicarbonate ( $\text{HCO}_3$ ) level in all the groups i.e. the normal control group (25.33±0.33), positive control group (25.33±0.33), negative control group (24.00±0.57), cancer + 300mg group (25.33±0.88), cancer + 600mg group (24.67±0.66), cancer + 1200mg group of extract (25.33±0.33) and cancer + 600mg+0.3mg of doxorubicin (25.33±0.33) were not significantly different ( $p>0.05$ ). The mean chloride ion ( $\text{Cl}^-$ ) value of the normal control group (101.33±1.85), positive control group (99.00±0.57), negative control group (98.67±0.33), cancer + 300mg group (102.67±2.33), cancer + 600mg group (99.67±0.88), cancer + 1200mg group of extract (99.67±0.66) and cancer + 600mg+0.3mg of doxorubicin group (101.00±1.15) were not significantly different ( $p>0.05$ ).

**Effect of Methanolic Leaf Extract of *Carica papaya* on Serum Level of IL-1 $\beta$  in Breast Cancer- Induced Wistar Rats**

The effect of methanolic leaf extract of *Carica papaya* on serum level of IL-1 (pg/ml) in Wistar rats induced with breast cancer is shown in table 5. The plasma level of IL-1 in the positive control group (6.23±0.59) was significantly higher than those in the normal control group (4.13±0.25), negative control group (4.43±0.05), cancer + 300mg group (4.14±0.23), cancer + 600mg group (3.78±0.13), cancer + 1200mg group of extract (2.82±0.50) and cancer + 600mg+0.3mg of doxorubicin (3.82±0.12) ( $p<0.05$ ). The mean values in the cancer + 1200mg of extract group, however, was significantly lower than normal control group, negative control group, cancer + 300mg group, cancer + 600mg group, cancer + 1200mg group of extract and cancer + 600mg+0.3mg of doxorubicin higher than ( $p<0.05$ ). Table 2 shows the phytochemical screening of methanolic leaf extract of *Carica papaya*.

**Table 2: Phytochemical Screening of Methanolic Leaf Extract of *Carica papaya***

Sample Dilution	Test	Result
Extract in water	Carbohydrate test	
	a. Molish Test	+
	b. Fehling Test	+
Extract in water	Saponins	
	a. Frothing Test	+
Extract in water	Tannins	
	a. Ferric chloride Test	+
Extract in water	Phenols	
	a. Ferric chloride Test	+
Extract in water	Flavinoid	
	a. Ferric chloride Test	+
	b. Alkaline Test	+
Extract in water	Proteins/Amino acid	
	a. Xanthoprotic Test	+
Extract in water	Cardiac Glycoside	
	a. Kellar-Kellani Test	+
	b. Kiddy Test	+
Extraction in chloroform	Steroids (triterphenoid)	-
	a. Salkawakis Test	
Extract in diluted Hydrochloric acid	Alkaloid	
	a. Mayer's Test	+
	b. Wegner's Test	+

**Key:** positive (+) sign indicates presence  
 Negative (-) sign indicates absent

**Table 3: Effect of Methanolic Leaf Extract of *Carica papaya* on Liver Biomarkers in Breast Cancer-induced Albino Rats**

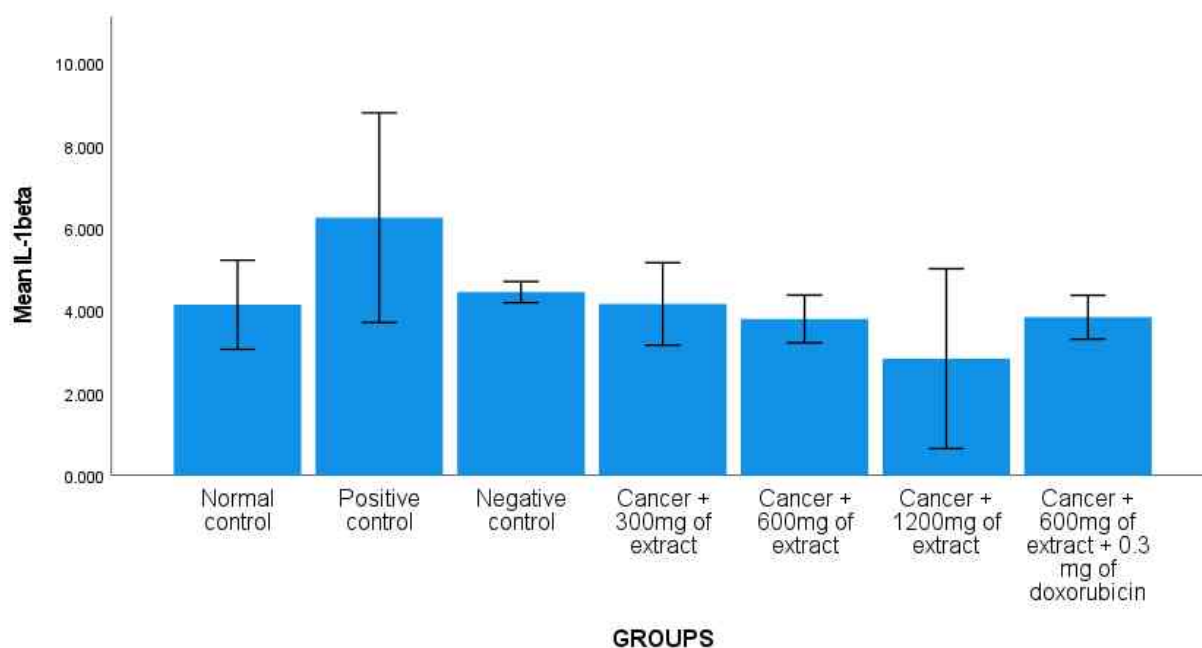
Groups	No of rats	AST (UI/L)	ALT (UI/L)	ALP (UI/L)	TP(g/dL)	ALB (g/dL)	DB (mg/dL)	TB (mg/dL)
I	3	7.00±1.15 <sup>a</sup>	8.00±0.57 <sup>a</sup>	89.33±0.88	68.33±0.33	38.33±0.33	0.20±0.00	0.73±0.03
II	3	10.00±0.57 <sup>abcd</sup>	9.33±1.45 <sup>a</sup>	88.00±2.30	68.67±0.33	38.33±0.33	0.20±0.00	0.76±0.06
III	3	10.33±0. <sup>33ac</sup>	11.33±0.33 <sup>ab</sup>	90.00±1.00	65.00±0.57	37.00±0.57	0.23±0.03	0.80±0.57
IV	3	9.33±1.20 <sup>ad</sup>	10.00±1.00 <sup>a</sup>	88.67±3.48	64.67±0.33	38.33±0.33	0.20±0.00	0.73±0.08
V	3	12.00±0.57 <sup>ae</sup>	10.33±1.52 <sup>ac</sup>	93.00±1.00	64.33±1.45	37.67±0.33	0.26±0.03	0.76±0.03
VI	3	13.67±0.66 <sup>ef</sup>	13.00±0.33 <sup>a</sup>	90.00±1.52	64.00±0.57	37.67±0.66	0.26±0.03	0.90±0.05
VII	3	9.00±0.00 <sup>a</sup>	12.33±0.66 <sup>ad</sup>	89.67±0.66	64.00±1.15	36.00±1.73	12.46±12.26	0.83±0.03
F-value		8.093	3.276	0.765	6.117	1.254	0.995	0.393
P-value		<0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05

**Key:** N=number of rats, G1=Normal Control, G2=Positive Control, G3= Negative control, G4= Cancer +300mg of extract, G5= Cancer + 600mg of extract, G6= Cancer +1200mg of extract, G7= Cancer +600mg of extract +0.3mg of doxorubicin. Values are expressed as mean ± SEM using Analysis of variance (ANOVA), mean values having different superscript in a column are significantly different, TP = Total Protein, Alb = Albumin, AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline Phosphatase, T. Bil = Total Bilirubin, D.Bil = Direct Bilirubin, u/l = International Unit/ Liter, mg/dL = Milligrams per deciliter, g/dL = grams per deciliter.

**Table 4: Effect of Methanolic Leaf Extract of *Carica papaya* on Kidney Biomarkers in Breast Cancer-induced Albino Rats**

Groups	No of Rats	Urea (mg/Dl)	Creatinine (mg/dL)	K+(mmol/L)	Na+(mmol/L)	Cl-(mmol/L)	HCO3 (mmol/L)
G1	3	4.83±0.12 <sup>a</sup>	0.76±0.03	4.33±0.13	145.00±0.57 <sup>a</sup>	101.33±1.85	25.33±0.33
G2	3	5.93±0.14 <sup>ac</sup>	0.86±0.03	4.73±0.03	141.00±1.00 <sup>b</sup>	99.00±0.57	25.33±0.33
G3	3	7.56±0.08 <sup>b</sup>	0.83±0.03	4.70±0.05	138.67±0.88 <sup>bc</sup>	98.67±0.33	24.00±0.57
G4	3	4.93±0.46 <sup>a</sup>	0.80±0.05	4.30±0.11	133.67±0.88 <sup>ab</sup>	102.67±2.33	25.33±0.88
G5	3	5.40±0.51 <sup>a</sup>	0.83±0.08	4.36±0.23	131.00±0.57 <sup>bd</sup>	99.67±0.88	24.67±0.66
G6	3	4.90±0.70 <sup>a</sup>	0.90±0.00	4.50±0.10	140.67±1.20 <sup>be</sup>	99.67±0.66	25.33±0.33
G7	3	6.26±0.20 <sup>ac</sup>	0.83±0.03	4.60±0.10	140.33±2.02 <sup>bf</sup>	101.00±1.15	25.33±0.33
F-value		6.552	0.833	2.306	3.823	1.209	1.130
P-value		<0.05	>0.05	>0.05	<0.05	>0.05	>0.05

**Key:** N=number of rats, G1=Normal Control, G2=Positive Control, G3= Negative control, G4= Cancer +300mg of extract, G5= Cancer + 600mg of extract, G6= Cancer +1200mg of extract, G7= Cancer +600mg of extract +0.3mg of doxorubicin. Values are expressed as mean ± SEM using Analysis of variance (ANOVA), mean values having different superscript in a column are significantly different, different, mg/dL = Milligrams per deciliter, mmol/L =milli moles per liter, Na+= Sodium, K+= Potassium, Cl- =Chloride, HCO3-=Bicarbonate



**Figure 1:** The Serum level of IL-1 $\beta$  in various groups treated with different doses *C. papaya* extract. Results are presented as Mean  $\pm$ SD, using Analysis of variance (ANOVA). G1=Normal Control, G2=Positive Control, G3= Negative control, G4= Cancer +300mg of extract, G5= Cancer + 600mg of extract, G6= Cancer +1200mg of extract, G7= Cancer +600mg of extract +0.3mg of doxorubicin.

## Discussion

This study shows that the leaf of *Carica papaya* contains many powerful phytochemicals such saponins, flavonoids, tannins, alkaloid, phenols which have anti-inflammatory and antioxidant property. This in line with the findings of Singh *et al.* (2019) who shows that the leaf of papaya contains many phytochemicals especially saponins, hydrolysable tannin, flavonoids, tannins, phenol, and alkaloids which have anti-inflammatory and antioxidant coupled with their immunomodulatory properties, and abilities to induce anti-proliferative and anti-apoptotic effects on cancer cells (Rahmat *et al.*, 2019).

In this study 30mg/kg of DMBA was used to induce breast cancer in the Wistar rats. The administration of DMBA successfully caused the development of tumour in the breast of the Wistar rats after 14days of induction. The tumour was noticed by palpating the breast and measuring the size of the tumour. DMBA has been validated as a useful and accurate model to induce tumours in mammary glands of rodent for years as it has been successfully used in many research to induce different kinds of tumour including breast cancer (Isabelle, 2021).

The study indicated a significant increase in AST in the positive control group, negative control group, 300mg, 600mg, 1200mg of extract group and 600mg + 0.3mg of doxorubicin groups when compared to normal control rats. This is in accordance with the findings of Surinder *et al.*, (2023) who discovered the serum concentration of AST in group with breast cancer is significantly higher than in those without. Also previous studies have found that liver function is poor in 92% of the patient diagnosed with breast cancer. This indicates that the increase is therefore due to breast cancer rather than the papaya extract because the serum concentration of AST is also increased in the positive control. Which is in accordance with the findings of Barine *et al.*, (2019) who observed that that the leaf extract of *C. papaya* has no significant effect on serum AST concentration. This result also agrees with the work of Nkiruka *et al.* (2024) who discovered that the leaf extract of papaya has no significant change in the AST when compared with the control group. This study is an indication of no hepatic damage caused by administration of leaf extract of *C. papaya* under study.



The study indicated a significant increase in ALT in the negative control group (cancer + doxorubicin group) and 600mg of extract + 0.3mg of doxorubicin groups when compared to normal control rats. This is in accordance with the findings of Hegazy *et al.* (2021) who discovered that treatment with doxorubicin significantly caused an increase in ALT. And this is also in accordance with the work of Doli *et al.* (2022) who observed a significant increment in ALT in DOX- treated rats when compared with normal control rats, indicating liver injury. However, no significant increase in ALT was observed when 300mg, 600mg and 1200mg of the extract was compared with normal control group. This study agrees with the work of Hicham (2021) who discovered that *C. papaya* leaf juice and aqueous extract are non-toxic even at high doses up to 2000mg/kg there was no significance difference between the normal control group and groups treated with different doses of *C. papaya* extract. This study is an indication that the increase in ALT is due to doxorubicin. ALT is an enzyme that helps in processing of protein problem in liver. Various liver disorders alter the blood vessels of these chemicals (Dorcas and Solomon, 2014). No significant difference was observed in ALP, ALB, total protein, albumin and direct bilirubin between treated groups, positive control and negative control group when compared to normal control which agrees with the findings of Nkiruka *et al.* (2024) who discovered that compared to the normal control, there is no significance difference in liver function parameters. However, the result of the analysis of liver enzymes disagrees with the findings of Audu *et al.* (2017) and Eyo *et al.* (2013), when they investigated the toxicity and effect of aqueous seed extract of *C. papaya* on liver biomarkers. Their study shows that ALP level is significantly higher and dose and time dependent. This disparity may be in part because different parts of the plant was used in both studies.

The study indicates significant increase in serum urea between negative control and 600mg of extract + 0.3mg doxorubicin group when compared to normal control which is in line with the findings of Catherine *et al.* (2021) who reports that doxorubicin raised plasma urea. However, there is significant increase in serum urea in negative control when compared with

extract + 0.3mg doxorubicin group. This is in line with other reports of doxorubicin-induced elevation in urea level (Chen *et al.*, 2016). However, there is significant decrease in serum urea level in the groups treated with extract when compared with negative control. The reduction in serum urea produced by the extracts is suggestive of their capacity to protect the nephrons. (Jambhulkar *et al.*, 2014) and thus preserve the glomerular filtration apparatus. The extract lowering of plasma urea may be due to their content of caffeic and chlorogenic acids, both of which had been reported to decrease plasma levels of kidney markers to near normal levels (Catherine *et al.*, 2021). No significant difference was observed in creatinine, K<sup>+</sup>, Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> between positive control and treated group when compared with normal control. This disagrees with the findings of Igbinovia *et al.* (2015) who reported that there is significant increase in kidney biomarker. The difference maybe in part because different part of the plant and doses were used in both studies.

The plasma level of IL-1 in the positive control group was significant higher than those in the normal control group, negative control group, cancer + 300mg group, cancer + 600mg group, cancer + 1200mg group of extract and cancer + 600mg+0.3mg of doxorubicin. This result in clash with the findings of Sanarto *et al.*, (2023) who observed that following treatment with leaf extract of *Carica papaya* the positive control exhibits the highest average expression IL-1 which occurs due to inflammation-driven carcinogenesis. But following treatment with three concentrations of *Carica papaya* (300mg, 600mg and 1200mg of extract) for 14days show a significant gradual decrease in IL-1 $\beta$  expression in the DMBA induced breast cancer in Wistar rats. The higher the concentration of papaya leaf extract the lower the IL-1  $\beta$  expression, The decrease happens because of the content of flavonoids, alkaloids, tannins and saponins as anti-inflammatory within the papaya leaf extract which can reduced IL-1 expression as a pro-inflammatory cytokine. This is in line with the findings of Sanarto *et al.* (2023) who observe that following treatment *Carica papaya* leaf extract the extract is capable of reducing IL-1 $\beta$  in dose dependent manner due to the presence

of saponins, hydrolysable tannin, flavonoids, tannins, phenol, and alkaloids in the leaf of papaya extract. This is also in line with the findings of Hyun *et al.* (2021) who reported that following treatment *Carica papaya* leaf extract the extract is capable of reducing IL-1 $\beta$  in dose dependent manner.

### Conclusion

The methanolic leaf extract of *C. papaya* has shown immunomodulatory potential effect in the downregulation of serum level of IL-1 $\beta$ . Moreso it has no toxic effect on the kidney and liver biomarkers. It is concluded that *Carica papaya leaf extract* may be used in the management of breast cancer patients.

### Recommendations

Frequent consumption of *C. papaya* should be encouraged in the larger society, and further investigations should be conducted to elucidate the underlying mechanisms by which the leaf extract of *C. papaya* exert its anti-cancer, immunomodulatory, renal and hepato-protective protectives in breast cancer patients.

### Conflict of Interest

This is no conflict of interest to declare

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