Original Research Article

Hepatoprotective properties of *Citrus aurantifolia* juice and *Carica papaya* seed extract mixture on Carbon Tetrachloride Induced Liver Injury in Albino Rats

ABSTRACT

Background: *Citrus aurantifolia and Carica papaya*, are commonly known as lime and paw-paw respectively, they are used in traditional medicine for the treatment of liver diseases and other various diseases. This study investigated the hepatoprotective properties of the aqueous extracts of *Citrus aurantifolia* juice and *Carica papaya* seed against carbon tetrachloride (CCl_4)- induced hepatotoxicity on the serum biochemistry and histomorphology of the liver of albino rats.

Materials and method: Twelve (12) female albino rats were used for this study. They were weighed and divided into four groups (A-D) of three rats each. Pre-treatments with oral doses of 200mg/kg body weight of the aqueous extracts for group C and 400mg/kg body weight for group D for ten days via oral gavage. Group A (Baseline control) and B (Negative control) received food and water for ten days preceding liver injury induction using subcutaneous administration of 3ml/kg b.wt. of CCl_4 mixed in equal parts with olive oil. Liver injury was induced in all rats except rats in Group A. Blood samples were collected from the rats and sera obtained were used for the determination of serum levels of Total bilirubin, Alanine transaminase (ALT), and Aspartate transaminase (AST). Upon sacrifice, under anesthesia, liver tissues were excised for histological processing and microscopy.

Results: Increased serum activities of total bilirubin, ALT and AST, in CCl_4-treated (negative control) rats were observed when compared with baseline control. However, pre-treatments with aqueous extract of *Citrus aurantifolia* juice and *Carica papaya* seed reduced the serum levels of the total bilirubin and ALT levels of rats in groups C and D. Microscopical examination of the liver showed centrilobular necrosis, ballooning degenerated and vacuolated hepatocytes in CCl_4-treated rats but improvement of the liver damage was observed in rats pre-treated with aqueous extract of *citrus aurantifolia* juice and *Carica papaya* seed.

Conclusion:Aqueous extracts of *Citrus aurantifolia* juice and *Carica papaya* seed possess hepatoprotective activities against CCl_4-induced hepatotoxicity in rats.

Keywords:-*Histomorphology, Citrus aurantifolia, Carica papaya, Liver, Hepatoprotective, Albino rats*.

INTRODUCTION

Background of study

Citrus aurantifolia is well-known for having a high concentration of vitamin C, a powerful antioxidant that is essential for collagen synthesis, immune system function, and the body's defense against dangerous free radicals. [1].Citrus aurantifolia includes a number of bioactive substances in addition to vitamin C, such as flavonoids and essential oils, which have been linked to anti-inflammatory, anti-cancer, and antioxidant properties. [2].These attributes have attracted the interest of researchers and health enthusiasts, making *Citrus aurantifolia* a subject of scientific investigation to uncover its potential health benefits beyond its delightful flavor. [3].

Similarly*, Carica papaya* is known for its versatility and nutritional richness. Papaya is a rich source of vitamins, particularly vitamin A and vitamin C, as well as essential minerals like potassium and magnesium. [4]. Additionally, Carica papaya has special enzymes like chymopapain and papain that are known for their ability to aid in digestion and perhaps have anti-inflammatory qualities. [4,5]. The fruit is a source of phytochemicals like beta-carotene and flavonoids, which have garnered attention for their possible roles in promoting skin health, reducing the risk of chronic diseases, and aiding in digestion.

One of the most significant organs involved in maintaining the body's homeostasis is the liver. Liver disorders are severe since the liver is the first organ to be exposed to various toxic, metabolic, neoplastic, and microbiological assaults. Exposure to these toxins, both acute and chronic, has a deleterious effect on the liver's fundamental functions.[6,7,8].Liver helps in detoxifying and metabolizing drugs, therefore a wide range of pharmaceutical and environmental substances have the potential to injure and cause serious complication.[6,7].

In order to assess the hepatoprotective impact of natural products, hepatotoxicants are used in experimental studies. The most common hepatotoxic agent used in experimental models of liver problems is carbon tetrachloride [ccl4]. Trichloromethyl radicals, an active metabolite of carbon tetrachloride, are the primary cause of the drug's hepatotoxic effects. When rats are given CCL4, their hepatic proteins oxidize more easily, causing a build-up of oxidized proteins in the liver. Many natural items have been tested for their ability to protect the liver from hepatotoxic chemicals like ccl4. [9,8,10].

MATERIALS AND METHODS

Collection of Plant Materials

Fresh fruits of *Citrus aurantifolia* (lime) were harvested from a seed-bearing plant and fresh seeds of *Carica papaya* were also collected from *Carica papaya* fruits from a farm settlement in Nsukka, Enugu state south Eastern, Nigeria. The *C.aurantifolia* fruit and *Carica papaya* seeds were identified by a Botanist at herbarium Department of Botany, Faculty of biological Sciences, University of Nigeria, Nsukka. The specimens were given identification numbers respectively (**UNH/05/0317C** and **UNH/04/0318E**).

Preparation of Fresh Juice and seed Extract

Seeds from *Carica papaya* fruits were washed thoroughly, dried under shade and ground into powder using a gasoline powered grinding machine.

Three hundred (300) grams of fresh lime fruits were peeled, washed and crushed in a fruit juice extracting machine. A muslin cloth was used to filter the resultant juice homogenate twice.

The crude aqueous extract was prepared by diluting 100ml of *citrus aurantifolia* juice with 400ml of water, the mixture was homogenized intermittently with a wooden stirrer for 2hours, 200g of the powdered seed of *Carica papaya* was dissolved in 2litres of distilled water, stirred intermittently and allowed to stand for sixty minutes, a muslin cloth was used to sieve the mixture, the filtrate and the prepared Carica papaya juice were thereafter stored in the refrigerator at (2-8°C) in leak-proof containers to be reconstituted when needed.

Ethical Consideration

Ethical approval for this study was sought and obtained from the Department of Animal Science, University of Nigeria Nsukka.

Experimental Animals

Twelve (12) female Albino rats weighing (100-135g) about 3 months old obtained from Animal House at the College of Medicine University of Nigeria Enugu Campus were used for this study. They were handled under standard conditions of temperature (27± 2^0C) and a 12-hour light, 12-hour dark cycle. The animals were housed in groups in metallic cages and were fed on standard commercial rat feed and clean water *ad libitum*. They were allowed for a period of two weeks for acclimatization, before the commencement of the study.

Experimental Protocol

The rats were weighed and divided into 4 groups A - D of three (3) rats each. Group A and B served as the Baseline and Negative control respectively while Group C – D served as the test groups. Group A and B received water while Groups C and D received 200mg/kg and 400mg/kg body weight of aqueous (*Citrus aurantifolia*juice and *Carica papaya* powdered seed*)* extract orally via oral gavage respectively for ten (10) days. All animals were handled in accordance to Institutional approved guidelines for the care and use of animals for scientific research set by National Institutes of Health on experiments involving the use of animals.

Induction of Liver Injury Using CCl_4

The albino rats were starved for twenty-four hours on Day Eleven before liver damage was induced. At the conclusion of the fasting period, rats in groups C and D were given 200 mg/kg and 400 mg/kg of papaya seed extract from Citrus aurantifolia and Carica. 45 minutes after the extract was administered orally, rats in groups B, C, and D were given 3 ml/kg body weight of CCl4 dissolved in equal parts of olive oil subcutaneously, causing liver damage. Rats in group A were given 10 ml/kg of normal saline orally. [10]

Following an hour of CCl4-induced liver injury induction in the rats in groups B, C, and D, all of the rats were sacrificed under chloroform anaesthesia, and their livers were removed, rinsed in normal saline, dissected, and the lesions in the liver tissues were photographed and scored using a digital camera.

Collection of Specimen for Biochemical Assays

Following a sixty-minute induction of liver injury, blood specimens were obtained by retro-orbital puncture of the medial canthus of the eye using a capillary tube into plain tubes while under chloroform anaesthesia. Following approximately 45 minutes of clot retraction, the blood specimens were centrifuged at 3000 rpm for 20 minutes, and sera were extracted from each sample for biochemical analysis. Standard commercial reagent kits were used to estimate the serum levels of ALT and AST as well as total bilirubin.

Tissues Processing and Microscopy

The excised liver tissues were then further preserved in 10% formol saline before being processed histologically utilising the paraffin wax embedding method for light microscopy analysis. An automatic tissue processor was used to remove water from the liver tissues and prepare them for wax impregnation. The tissues infused with paraffin wax were "blocked out" before the sections were cut using the Hertz 150, Cambridge type rotary microtome. The Haematology and Eosin (H and E) staining method, as outlined by [11], was used to create five (5) um tissue slices, which were then further stained. Using an Olympus binocular microscope with an integrated illumination system, the sections were inspected.

Photomicrography

Examination of stained sections was done using a shift®N x 1000 digital Camera attached to a Magnus®trinocular microscope.

Statistical Analyses

The statistical analysis was performed using statistical Package for Social Sciences (SPSS) version 26.0. Data obtained were expressed as mean ± standard error of mean (SEM). One - way analysis of variance (ANOVA) and unpaired two-tailed student's t-test were used to determine the difference among the groups, and the Post Hoc for multiple comparison. The level of significance was considered at $P < .05$

RESULTS

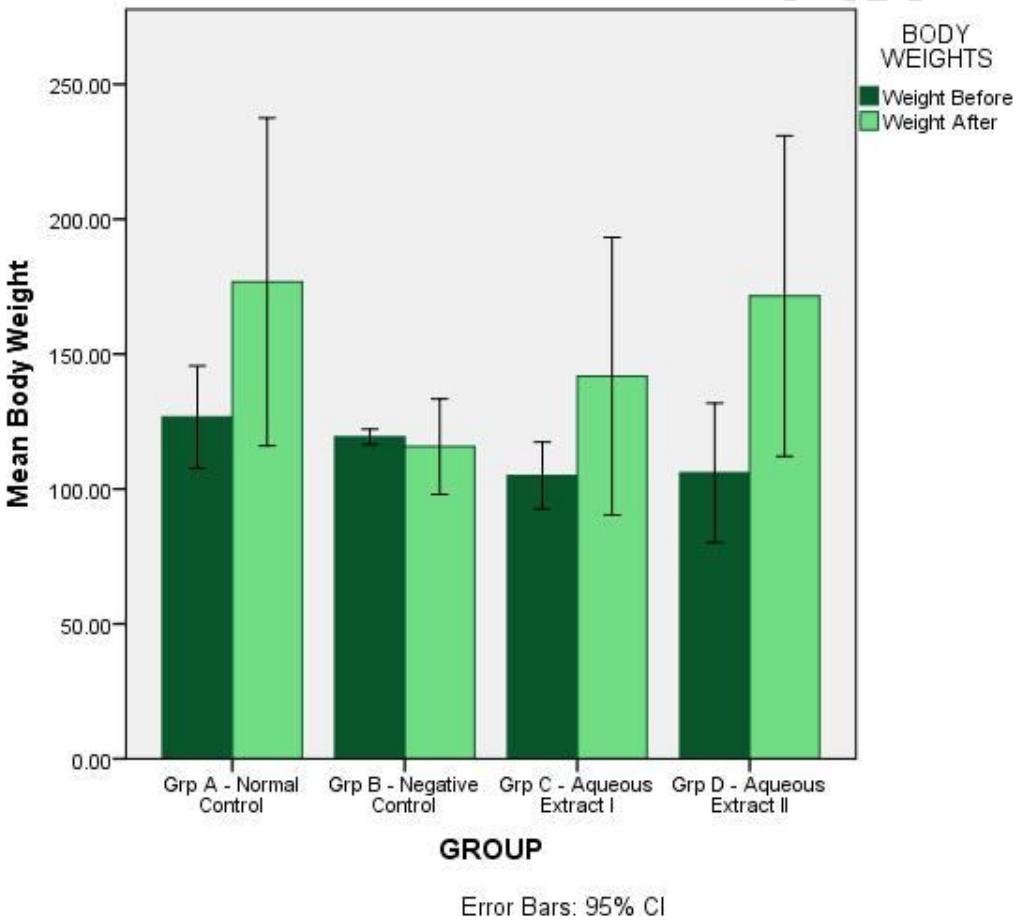

Fig. 1: Bar chart shows an appreciable weight increase in group A C and D. And weight lost in group B.

Biochemical Assays

Table 1: Comparison of the Liver Enzymes with the Controls

Groups	Total bilirubin (mg/kg) Mean±SEM	ALT(µ/L) Mean±SEM	AST(µ/L) Mean±
A(Baseline control)	0.90±0.29	25.33±4.91	36.00±2.89
B(Negative control)	0.91±0.10	36.33±15.51	39.33±4.81
C(Low dose aqueous extract)	0.70±0.30	15.33±1.67	45.33±4.81
D(High dose aqueous extract)	0.88±0.14	21.00±2.31	41.33±5.17
F-ratio	0.33	1.16	1.01
P-value	.81	.38	.44

Photomicrographs

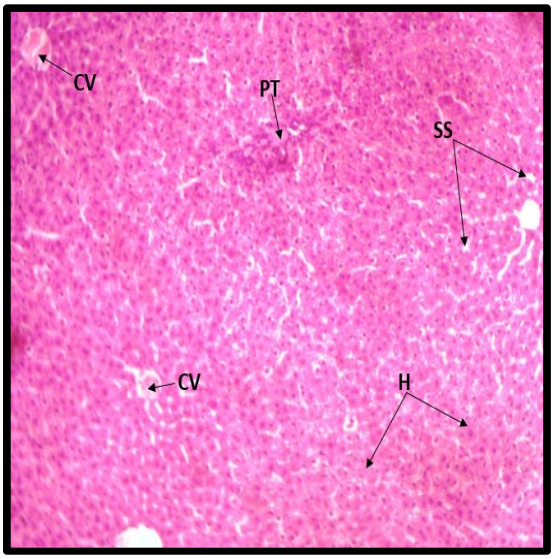

Plate 1:Light photomicrograph of Liver section from normal control rat (Group A) showing normal histoarchitecture of the hepatic tissue. Normal features including central veins (CV), hepatocytes (H), portal tracts (PT) and sinusoidal spaces (SS) appear normal. (Stain: H&E; Mag: x100)

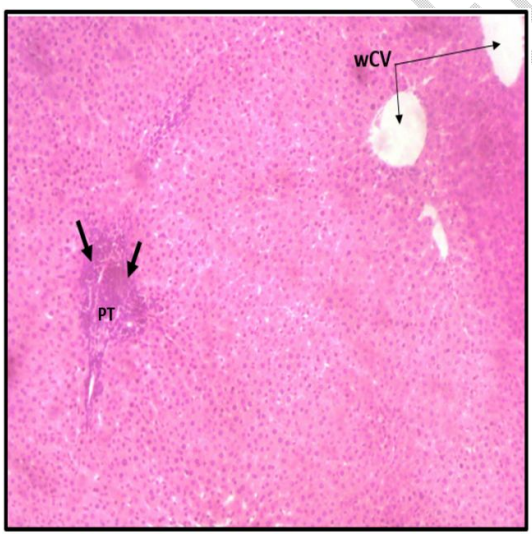

Plate 2:Light photomicrograph of Liver section from rat treated with CCl4 only (Negative Control) showing some obvious histomorphological alteration. Features observed include: markedly enlarged portal tracts with inflammatory cellular infiltration (PT); necrosis and degeneration of hepatocytes surrounding the portal tracts (thick arrows); central veins are mildly widened(wCV). (Stain: H&E; Mag: x100)

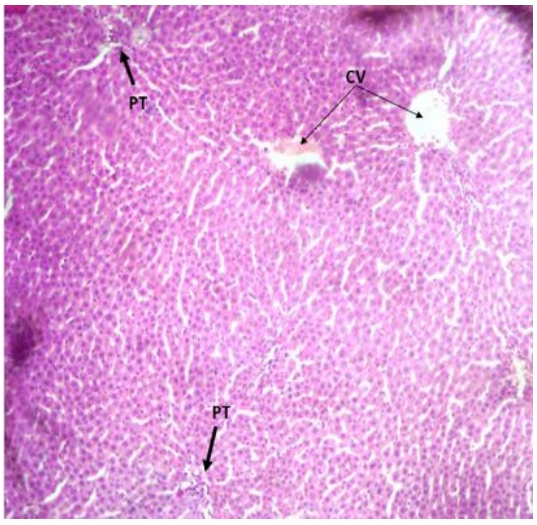

Plate 3:Light photomicrograph of Liver section from rat treated withlow dose of CA-CP Aqueousextract (Group C) prior to liver injury induction with CCl4 showing intact tissue parenchyma. The hepatocytes around the central veins (CV), portal tracts (PT) and mid-zonal areas appear intact with no obvious lesion.(Stain: H&E; Mag: 100)

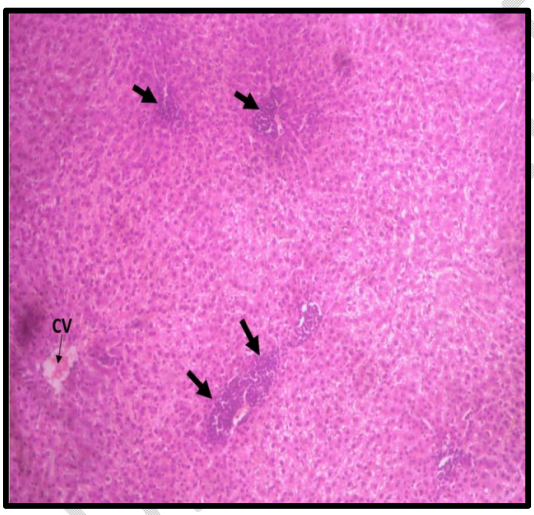

Plate 4:Light photomicrograph of Liver sectionfrom rat treated with high dose of CA-CP Aqueous extract (Group D) prior to liver injury induction with CCl4 showing evidence of tissue disruption. Marked inflammatorycellular infiltration and necrosis are noted at periportalregions(thick arrows). The central veins (CV) and centrilobular hepatocytes reveal no obvious histomorphological alteration (Stain: H&E; Mag:x100).

DISCUSSION

Due to its numerous health benefits, citrus aurantifolia is widely produced as both sources of food and of medicine. When used together to treat liver diseases including hepatitis, lime and paw-paw seed extracts have demonstrated important nutritional and therapeutic benefits. [12]. This study investigated the hepatoprotective effect of *Citrus aurantifolia* juice and paw-paw seed mixture against carbon tetrachloride (CCL_4) induced liver injury by estimating liver enzymes and liver histology of Albino rats.

When comparing the weights of the rats at the end of the experiment to their weights prior to the trial, the study's findings showed that the rats in groups A, C, and D had significantly increased their body weights, whereas the rats in group B had decreased. A prior work on "Disturbances in Calcium and Zinc Homeostasis during testicular damage induced by Citrus aurantifolia Juice in wistar Rats" was contradicted by this research.[13].

P-synephrine and citrus aurantifolia extracts were utilised in another study to improve energy, focus and cognition, hunger control, sports performance, and weight loss/weight management. [14] refute the results of the current investigation as well.

On the other hand, current study data demonstrate the efficiency of carbon tetrachloride in causing hepatotoxicity in experimental animal, as seen by the ballooning degeneration and vacuolation of hepatocytes surrounding the central veins. Previous studies have demonstrated similar histological changes following ccl4 treatment.[15]. However, pretreatment with aqueous extracts of Carcia papaya seed and Citrus aurantiofolia juice showed that the rats' liver tissue parenchymal was only minimally retained. [15]. The phytochemicals included in the plant material may have a single or combination effect on the tissue preservation seen in this study.

The common method for assessing the degree of liver damage is to measure its markers which includes Total bilirubin, serum, Alanine Transferase (ALT) and Aspartate Transaminase (AST). ALT is a more specific enzyme that indicates liver disease. [16]. Increased level of these serum liver markers was observed after treatment with ccl4 in the present study, which correlates with the histopathological report indicating liver injury in rats that served as the negative control when compared to rats in groups C and D which were pre-treated with aqueous extracts of *Citrus aurantiofolia*juice and *Carcia papaya* seed mixture, compared to rats treated only with ccl4, levels were found to be reduced. This demonstrates that the effects of the hepatotoxicants-CCL4, has been alleviated.

A further investigation titled "Modulatory potentials of aqueous leaf and unripe fruit extracts of Carica papaya Linn. (Caricaceae) against carbon tetrachloride and acetaminophen-induced hepatotoxicity in rats" [17]. further demonstrates a drop in liver enzyme levels in the therapy groups.

Furthermore, the hepatoprotective activity of *Carica papaya* seed extracts has been reported against carbon tetrachloride induced hepatic damage in rats [15].Another study on "Abrogation of carbon tetrachloride (CCl_4) induced hepatotoxicty by arogyavardhani - a multi herb supplement, in wistar rats" shows high level of liver enzymes on the group treated with only CCL_4. [15].

CONCLUSION

In conclusion, the biochemical and histopathological results obtained from this study confirm the hepatoprotective effect of the aqueous extracts of *Citrus aurantifolia* juice and *Carica papaya* seed against carbon tetrachloride (CCl_4) induced liver damage in experimental rat model.

REFERENCES

1. Habeeb H, Thoppil JE. Vitamin C and Citrus peels—a treasure chest for healthy life. InHerbal Formulations, Phytochemistry and Pharmacognosy 2024 Jan 1 (pp. 401-411). Elsevier.

2. Jain S, Arora P, Popli H. A comprehensive review on Citrus aurantifolia essential oil: its phytochemistry and pharmacological aspects. Brazilian Journal of Natural Sciences. 2020 Jul 25;3(2):354-.

3. Al-Snafi AE. Nutritional value and pharmacological importance of citrus species grown in Iraq. IOSR Journal of Pharmacy. 2016;6(8):76-108.

4. Daagema AA, Orafa PN, Igbua FZ. Nutritional potentials and uses of pawpaw (Carica papaya): A review. Eur. J. Nutr. Food Saf. 2020;12:52-66.

5. Babalola BA, Akinwande AI, Otunba AA, Adebami GE, Babalola O, Nwufo C. Therapeutic benefits of Carica papaya: A review on its pharmacological activities and characterization of papain. Arabian Journal of

6. Ahmed O, Robinson MW, O'Farrelly C. Inflammatory processes in the liver: divergent roles in homeostasis and pathology. Cellular & Molecular Immunology. 2021 Jun;18(6):1375-86.Chemistry. 2024 Jan 1;17(1):105369.

7. Lian ZX, Li L. The liver as a lymphoid organ. Liver Immunology: Principles and Practice. 2020:17-33.

8. Sufyan R. *Exploring Antioxidant and Anti-Inflammatory Responses in CCL4-Induced Liver Injury Mouse Model* (Doctoral dissertation, Quaid I Azam University Islamabad).

9. Babu S, Ranajit SK, Pattnaik G, Ghosh G, Rath G, Kar B. An Insight into Different Experimental Models used for Hepatoprotective Studies: A Review. Current Drug Discovery Technologies. 2024 Jul 1;21(4):80-91.

10. Wu Y, He Y, Wang R, Zhao X. Preventive effect of flavonoid extract from the peel of Gonggan (Citrus reticulata Blanco Var. Gonggan) on CCl4-induced acute liver injury in mice. Journal of Inflammation Research. 2021 Oct 5:5111-21.

11. Hoque MZ, Keskinarkaus A, Nyberg P, Seppänen T. Stain normalization methods for histopathology image analysis: A comprehensive review and experimental comparison. Information Fusion. 2024 Feb 1;102:101997.

12. Maqbool Z, Khalid W, Atiq HT, Koraqi H, Javaid Z, Alhag SK, Al-Shuraym LA, Bader DM, Almarzuq M, Afifi M, Al-Farga A. Citrus waste as source of bioactive compounds: Extraction and utilization in health and food industry. Molecules. 2023 Feb 8;28(4):1636.

13. Izah SC, Richard G, Odubo TC. Citrus aurantifolia: Phytochemical Constituents, Food Preservative Potentials, and Pharmacological Values. InHerbal Medicine Phytochemistry: Applications and Trends 2023 Nov 28 (pp. 1-26). Cham: Springer International Publishing.

14. Gad MZ, Azab SS, Khattab AR, Farag MA. Over a century since ephedrine discovery: an updated revisit to its pharmacological aspects, functionality and toxicity in comparison to its herbal extracts. Food & Function. 2021;12(20):9563-82.

15. Azubuike NC, Onyemelukwe AO, Maduakor UC, Onwukwe OS, Ezeh AC. Hepatoprotective effects of the leaf extracts of Cassia occidentalis against carbon tetrachloride-induced hepatotoxicity in albino rats. Pharmacologyonline. 2018;3:68-74.

16. Jain P, Batta AK, Singh P. Comparative study of serum levels of gamma-glutamyltransferase, Aspartate aminotransferase (AST), Alanine Transaminase (ALT), AST: ALT, and bilirubin in patients with chronic hepatitis. Indian J. Med. Biochem. 2022 Sep;26(3):74.

17. Awodele O, Yemitan O, Ise PU, Ikumawoyi VO. Modulatory potentials of aqueous leaf and unripe fruit extracts of Carica papaya Linn.(Caricaceae) against carbon tetrachloride and acetaminophen-induced hepatotoxicity in rats. Journal of Intercultural Ethnopharmacology. 2016 Jan;5(1):27.