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Research Article

Biochemical Impact of Carica Papaya (Pawpaw) leaves Extract and Ruzu Bitters on Hematology and Brain Histology in Sprague Dawley Rats

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Abstract

Plant-based products have been utilized for nutritional and medicinal purposes for decades. Although the reported benefits of *Carica papaya* (Pawpaw) leave extract, its role in hematology, brain histology, and the possible side effect are still areas of research deliberation. Thirty (30) male Sprague Dawley rats, divided into three groups, were fed on rat chow and normal saline, *Carica papaya* leaves extract and Ruzu bitters, respectively. Blood chemistry, hematology, and brain histology were assayed to ascertain their effects on brain structure and biochemical changes. White blood cells, hemoglobin, red blood cells, platelets, and packed cell volume were carefully evaluated. In the sub-chronic test, there were no significant changes in PCV (%) in the papaya extract and Ruzu bitters group, relative to the control. There was a significant increase in hemoglobin levels in the papaya and Ruzu bitters groups. *Carica papaya* leaves extract and Ruzu bitters significantly increased certain serum biochemistry parameters ($p < 0.05$), compared to the control group. Our study revealed that *C. papaya* leaves extract possess an immunomodulatory effect and did not show any

detrimental effect on the brain histology, liver, and general well-being, unlike Ruzu bitters. The neuroprotective effect of the extract is apparent from the intact brain structure of treated rats compared to the other group, ($p < 0.05$). The hydro-ethanol leaf extract of *Carica papaya* possesses neuroprotective, anti-inflammatory, anti-hemolytic and immunomodulatory effects compared to the Ruzu bitters. However, both extracts' long-term administration should be taken cautiously and further investigated.

Keywords
Carica papaya; Ruzu bitters; phytotherapy; histology; hematology; antioxidant activities; blood chemistry

1. Background

Oxidative stress is a common pathway to all diseases and aging. The endogenous antioxidant defense system is often compromised due to inevitable endogenous and exogenous stress factors. Antioxidants from natural products could mitigate these effects. Hence, they prevent diseases, delay the onset of aging, and, most importantly, neutralize the toxic effects of xenobiotics, drugs and other oxidative stress inducers [1-3]. Natural products have been reported to ameliorate the devastating effect of drugs due to their antioxidant potency. Therefore, they prevent chronic diseases [4-6].

The Pawpaw plant (*Carica papaya* L.) belongs to the family *Caricaceae*. The fruit, leaves and seeds have a wide range of nutritional and medicinal properties. The fruit produces the enzyme papain, which aids digestion. The leaves are rich in beneficial phytoconstituents, potent antioxidants of biological importance [7]. Papaya leaves extract possesses antimicrobial activity and has been clinically used in chemotherapeutic-induced thrombocytopenia [8, 9]. An experimentally induced mammary tumor has been successfully cured using papaya leaf extract [10].

Moreover, due to the health-promoting effects of most plant-based products [11], the oral use of bitters which are poly-herbal formulations, in the form of herbal cocktail mixtures, has emerged over the years. In recent times, these bitters, such as Swedish bitters, Yoyo bitters, Alomo bitters, and Orijin, to mention a few, are chiefly consumed for their medicinal benefits. However, very little is known about the brain histology and biochemical and hematological effects of consuming Ruzu bitters, recently introduced into the Nigerian market.

Various bioactive drug components are obtained from medicinal plants as reported by the World Health Organization, these medicinal herbs are the most common type of alternative medicine [12]. Its prescriptions and natural remedies are a constant source of medication in treating different ailments. *Carica papaya* was reportedly an efficient anti-microbial agent and is essentially valued for its antibacterial activity [13]. Unfortunately, limited scientific evidence exists, regarding the safety and efficacy to back up the continued therapeutic application of these remedies. A thorough scientific investigation of these medicinal plants will go a long way in validating their domestic and long-term usage [11].

Most bitters have anti-inflammatory properties, such as Alomo bitters [14]. Ruzu bitters, which is also one of the poly-herbal formulations of medicinal importance in Nigeria have their

constituents to include; *Uvaria chamae* (20%), *Curculigo pilosa* (40%) and *Citrullus colocynthis* (40%). These three components, alongside other constituents, have been combined in a way that delivers the best value to human health and wellness. Ruzu herbal bitters, a unique blend of active ingredients, have been locally used in the management and control of various health-related problems such as diabetes, weak erection, typhoid fever, vaginal discharge, menstrual anomalies, high blood pressure, malaria, back and waist pain, fibroid tumor, infertility in men and women, syphilis, weight-related ailments and obesity. However, no research has verified these claims. *Carica papaya* L papaya, also known as papaw, Pawpaw, chickpea, mama, and melon tree, is highly cultivated because of the value of its leaves, shoots and fruits. The leaves are sometimes boiled as a vegetable, and taken as a herbal tea infusion, and the papaya fruit juice is enjoyed as a popular beverage. Papain, the bioactive enzyme in *Carica papaya,* is a highly beneficial pro-digestive agent.

Certain inflammatory conditions such as arthritis, rheumatism, asthma, malaria and wounds, may be managed using the *Carica papaya* leaf extract [15]. The leaves are rich in nutritive phytochemicals of ethnomedicinal use [16]. They have been reported to possess a gastric protective effect, due to the synergistic effect of the plant components [17]. *Carica papaya* extract has increased platelet counts in patients suffering from dengue fever [18]. A recent study has also used its black seed extract to manage prostate cancer [19]. However, little is known about the acute, sub-chronic toxicity and hematological effects of the *Carica papaya* leaves extract and Ruzu bitters, a locally consumed detox. These two are being compared in this study to verify the efficacy of the claims and beliefs about the beneficial effects of the bitters, which are not yet verified. Therefore, the main goal of the present study is to investigate the acute, sub-chronic toxicity and hematological effects of *Carica papaya* leaves extract and Ruzu bitters.

2. Materials and Methods

2.1 Herbal Bitters

Ruzu Herbal Bitters (natures pure marvel, 100% natural, no preservatives), used in the present study, is a product of Ruzu Natural Health Products and Services. It was obtained from Timotech Pharmacy, Lagos State, Nigeria.

2.2 Plant Material

Fresh leaves of *Carica papaya* were harvested from a vegetable garden at Yaba, Yaba Local Government Area of Lagos State, Nigeria. The specimen was authenticated by Mr. Nodza G. I. of the Botany Department, University of Lagos, Nigeria. A voucher specimen (No.7687) was deposited in the University's herbarium.

2.3 Preparation of Plant Extract

The fresh leaves of *Carica papaya* were dried under a shade and crushed into powder with an electrical mill. The dried leaf powder was soaked in 50% ethanol for 48 hours and filtered using Whatman no.1 filter paper. The filtrate obtained was stored at 4°C until further use.

2.4 Laboratory Animals

Thirty (30) healthy male Sprague Dawley rats were used in the present study. Their age range is 8-10 weeks old, and their weight is within the range of 110-145 g. They were obtained from the animal house of the College of Medicine, University of Lagos, Idi Araba, Lagos State, Nigeria. The experimental animals were maintained on a regular rat chow diet, under standard humidity conditions, temperature and a 12-hour light/dark cycle. They were acclimatized for a week before the commencement of the study. Animal handling was by the general guidelines for handling research animals, in line with the NIH Guidelines for the Care and Use of Laboratory Animals. This research was approved by the Health Research Ethical Committee, College of Medicine, University of Lagos, Lagos State, Nigeria. The ethical approval document number is: HREC/CMUL/2018/0018.

3. Methods

3.1 Mode of Administration

The thirty (30) rats were divided based on their body weight into three (3) groups comprising 10 rats per group, with an average body weight of 127.5 ± 17.5 g. The rats were housed in plastic cages with wire gauze as a covering to allow proper ventilation. Wood shavings were used as bedding. The animals had free access to food and water *ad libitum.* Animals in the control group received normal rat chow and water. The second and third groups of animals were administered 2 mL of Pawpaw leaves extract and Ruzu herbal bitters orally, with the aid of a cannula every other day. This concentration was chosen based on our preliminary investigation and a previously reported work [20]. Locally, people consume papaya leaf extract as a tea infusion. The administration of the herbal bitters and extract lasted 7 days and 14 days, representing the acute and chronic test, respectively.

3.2 Body Weight

The body weight and feed intake of rats were monitored, measured and recorded every other day for two (2) weeks.

3.3 Mortality and Clinical Signs

During the two weeks of administering the extract and herbal bitters, the experimental animals were observed for clinical signs and mortality patterns, before and after administration. This is to ascertain the safety of the chosen concentration of the papaya extract and herbal bitters.

3.4 Collection of Blood Samples

After the 7^{th} and 14^{th} day of papaya extract and herbal bitters supplementation, blood samples were collected via ocular puncture with capillary tubes. Samples were stored in a lithium heparin bottle. The blood samples were centrifuged at 3000 rpm for 30 min to separate the plasma (the lower part) into another lithium heparin bottle. The serum (the upper part) was separated into a plainly labeled bottle.

3.5 Blood Chemistry Test

The following parameters were determined colorimetrically by employing the standard ready-to-use kits and methods of Human (HUMAN Gesellschaft fűr Biochemica und Diagnostica mbH, Germany): aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, urea, and amylase. The manufacturer's instructions for each biochemical parameter were strictly followed during the investigations, using the Randox kit [21, 22].

3.6 Hematology

The packed cell volume (PCV) and hemoglobin (Hb) estimation were carried out using the microhematocrit and cyan-methemoglobin methods of Baker and Silverton. The methods of Baker and Silverton and Jain were employed to determine the total leukocyte counts (TLC). In contrast, the longitudinal method of Baker and Silverton, provided a good assay for the differential leukocyte count (DLC). The determination of white blood cells and some white blood cell differentials were measured using the improved Neubauer counting chamber [23].

3.7 Antioxidant Activities

Liver tissue was extracted in 5 mL of phosphate buffer; 0.5 g of the liver was mashed in a laboratory mortar and pestle. It was centrifuged at 3500 rpm for 5 min, the solution was decanted and the supernatant was recovered. 1 mL of the extracted tissue was drawn into test tubes using a micropipette. The sample was used for the *in vivo* antioxidant activity assays. The effect of extracts on laboratory animals is an in vivo assay.

3.8 Tissue Protein Determination Using Biuret Method

Four (4) mL of Biuret reagent was added to the liver sample and mixed thoroughly. The absorbance was read at 540 nm against a reagent blank which contained 1 mL of water and 4 mL of Biuret reagent [20].

3.9 Tissue Lipid Peroxidation Determination (MDA Assay)

One (1) mL of the liver sample was measured and poured into a clean labeled test tube, and two (2) mL of TBA (thiobarbituric acid) were added and boiled for 15 min; it was left to cool down after boiling. Absorbance was read at 532 nm against a blank that contained 1 mL of water and 2 mL of TBA [24].

3.10 Catalase Activity Assay

Exactly 0.1 mL of the liver sample was measured with a micropipette into the test tube, 1 mL of phosphate buffer and 0.5 mL of hydrogen peroxide were added and left to stand for 60 s. After that, 2 mL of dichromate reagent was added and mixed thoroughly. The blank and control samples, contained 1.5 mL of phosphate buffer with 2 mL of dichromate reagent and 0.5 mL of hydrogen peroxide with 2 mL of dichromate reagent respectively. All the test tubes were left to boil for 10 min. Absorbance was read at 570 nm against the blank, and then the control [25].

3.11 Reduced Glutathione (GSH) Determination on the Tissue

One (1) mL of the liver supernatant sample was measured into test tubes 1 mL of TCA (trichloroacetic acid) was added with 1 mL of phosphate buffer and 0.5 mL of DTNB (5,5`dithiobis-2-nitrobenzoicacid). Absorbance was read at 412 nm [26].

3.12 Histology

Brain samples were fixed in formalin in universal bottles, hematoxylin and eosin-stained. 5 mm thick sections were used and examined by light microscope. The light microscope's specifications include two eyepieces (binocular) and the total magnification is 100×. Reports were obtained and micrographs (magnification: 100×) were developed.

3.13 Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 6 statistical package (GraphPad Software, San Diego CA, USA). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. All the results were expressed as mean ± SD for 10 rats in each group and were considered statistically significant at a 95% significance level ($p < 0.05$).

4. Results

The increasing body weights were adequately observed to know the biochemical impact of the hydro-ethanol leaves extract of *Carica papaya* and Ruzu bitters in Sprague Dawley rats, and histology of brain sections was assessed. Acute and sub-chronic evaluations of hematologic parameters, blood biochemistry and *in vivo* antioxidant capacity of the leaf extract were equally evaluated.

4.1 Effect of Carica Papaya Leaves Extract and Ruzu Bitters Supplemented Diet on Body Weight

There was a significant increase in body weight of *the Carica papaya* administered group ($p < 0.0001$) compared to the Ruzu bitters group, which had no significant change in body weight (Figure 1).

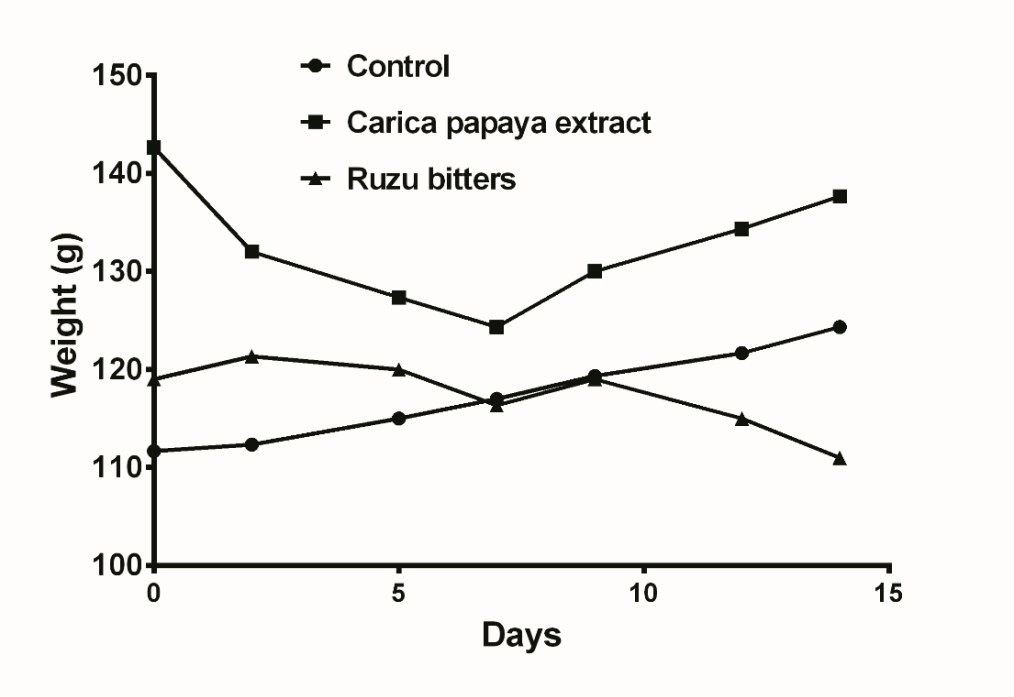

Figure 1 Effect of *Carica papaya* leaves extract and Ruzu bitters on body weights of animals. There was a significant increase in body weight of papaya extract group of rats ($p < 0.0001$) and no significant changes in the Ruzu bitters administered rats ($p = 0.9997$) compared to the control.

4.2 Effect of Carica Papaya Leaves Extract and Ruzu Bitters on The Histological Section of Rat Brain Tissue and Biochemical Parameters

No abnormalities were observed in the treated animals' brain tissues (Figure 2). The mean white blood cell WBC (cmm) and hemoglobin HGB (g/dl) levels in the serum of treated groups of rats revealed a significant increase compared to the control. The sub-chronic administration test revealed an increase in WBC levels in both treated groups compared to the control. The acute administration revealed a decrease in HGB (g/dl) levels in the papaya group compared to the other groups (Figure 3). Effect of *Carica papaya* extract and bitters on mean PCV (%) and mean RBC ($\times 10^{12}$/L) level in acute and sub-chronic administration revealed very mild changes in the groups, both at the sub-chronic and acute levels (Figure 4). Effect of extract and bitters on the mean platelet level (/cmm) and mean neutrophil (%) level in acute and sub-chronic administration revealed an increase in the mean platelet levels in both treatment groups in the acute test. At the same time, no changes were observed in the sub-chronic test. There was a significant decrease in the neutrophil levels of treatment groups in the acute and sub-chronic tests (Figure 5).

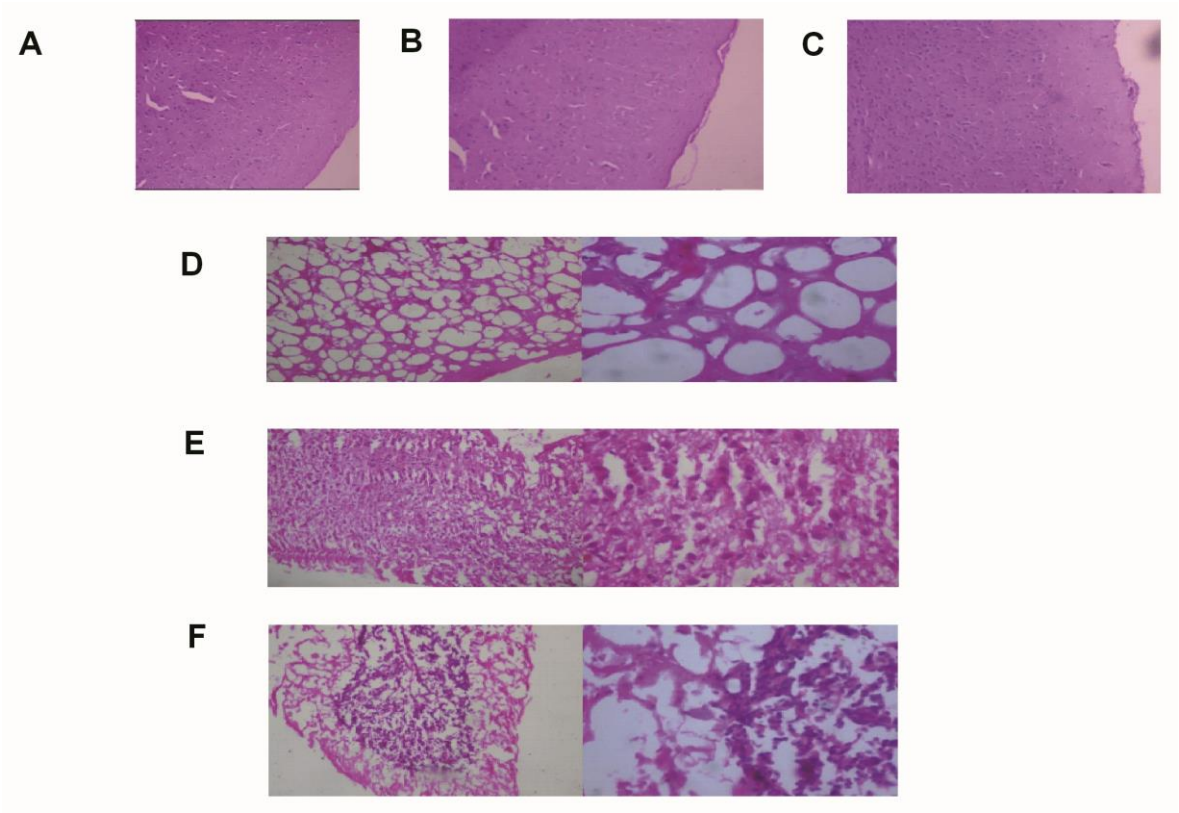

Figure 2 Effect of *Carica papaya* leaves extract and Ruzu bitters on the histological section of rat brain tissue. **(A)** Micrograph of rat brain from the control group, **(B)** Ruzu herbal bitters group, and **(C)** Pawpaw leaf extract group after 7 days (100×; H & E). **(D)** Micrograph of the brain from the control group **(E)** Ruzu herbal bitters group **(F)** Pawpaw leaf extract group after 14 days (100×; H & E). Figure **A, B and C** showed the histological sections of brain tissue with neuronal cells on a background of neutrophil. No abnormalities are seen. Normal brain feature. Figure **D**, brain section of the Ruzu bitters group above, showed the histological section revealing flattened ductal epithelial cells enclosing central empty (fat filled) spaces. They form ducts. No abnormalities are seen. Figure **E** above showed the histological section revealing aggregates of cells with large pleomorphic hyperchromatic nuclei not disposed as ducts but as sheets, an abnormal brain feature. Figure **F**, the brain section of the *C. papaya* leaves extract group above, showed the histological section revealing flattened ductal epithelial cells enclosing central empty (fat filled) spaces at the periphery where they form ducts. Centrally, cells are more compact with small nuclei. No abnormalities seen, normal brain feature.

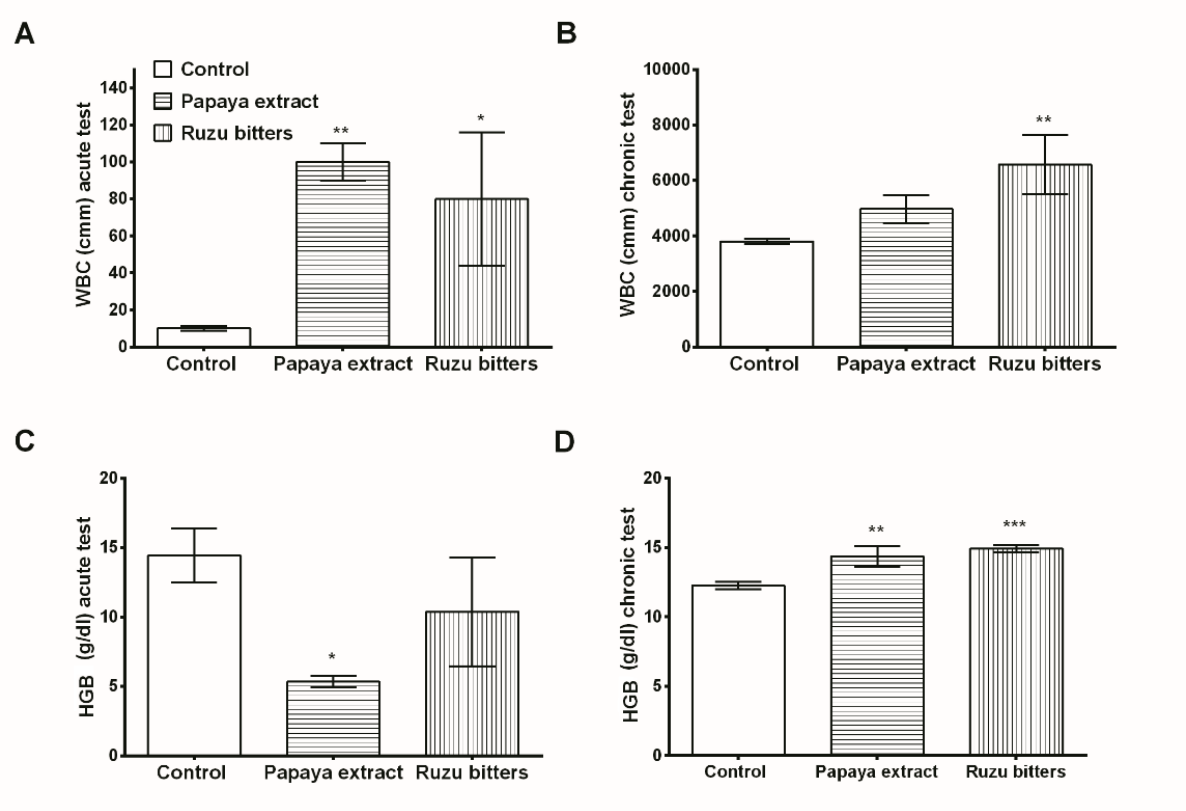

Figure 3 Mean white blood cell WBC (cmm) and HGB (g/dl) levels of treated groups of rats in the acute and chronic test respectively. Effect of extract and bitters in acute and sub-chronic administration. **(A)** There was a significant increase in WBC levels in papaya extract and bitters group compared to the control, p = 0.0040 and 0.0133 respectively in the acute test **(B)** There was a significant increase in WBC levels in papaya extract and bitters group compared to the control, p = 0.1386 and 0.0046 respectively in the sub-chronic test, **(C)** There was a significant decrease in hemoglobin levels in papaya extract (p = 0.0081) and not in bitters group (p = 0.1623) compared to the control, in the acute test **(D)** There was a significant increase in hemoglobin levels in papaya extract (p = 0.0032) and bitters group (p = 0.0009) compared to the control, in the acute test as evaluated by Dunnett's post hoc test.

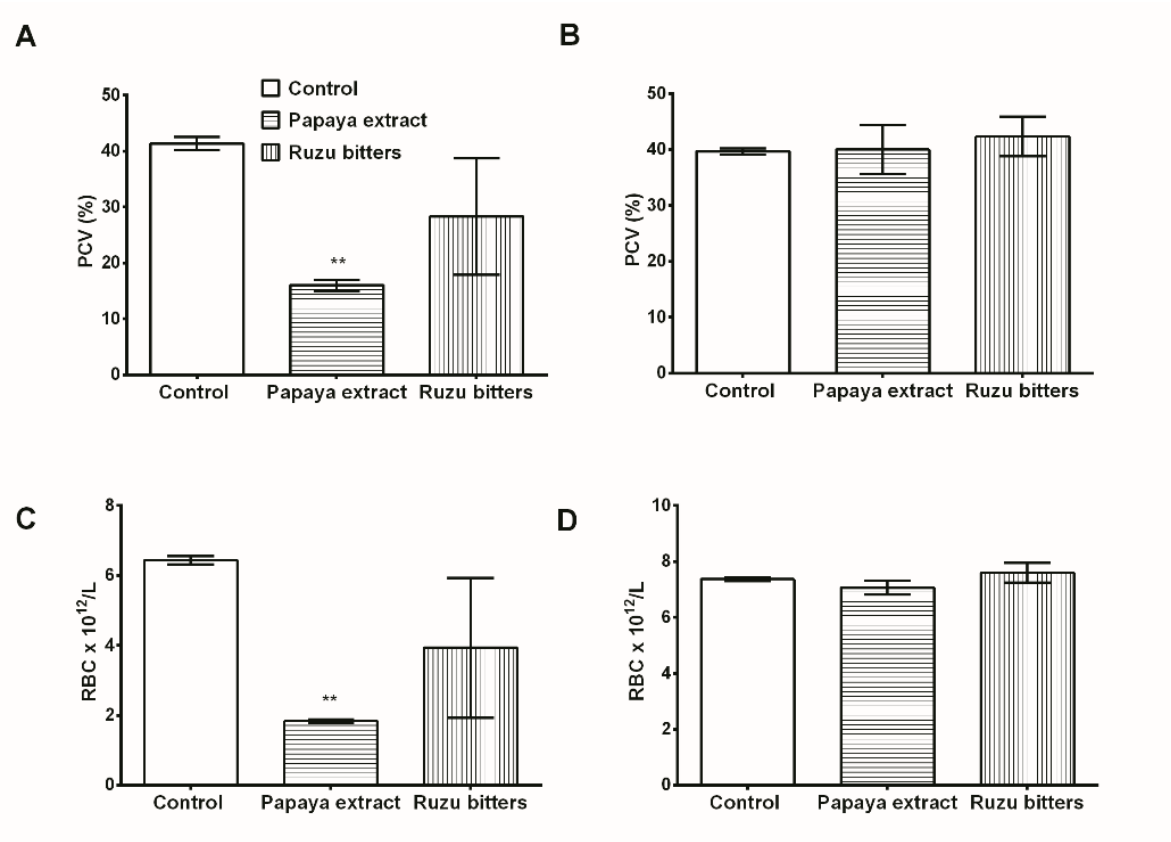

Figure 4 Mean packed cell volume PCV (%) and mean red blood cell RBC (× 10^{12}/L) level of different groups in acute and chronic test respectively. Effect of extract and bitters on mean packed cell volume PCV (%) and mean red blood cells RBC (× 10^{12}/L) level in acute and sub-chronic administration. **(A)** There was a significant decrease in packed cell volume PCV (%) in papaya extract and no changes in the bitters group compared to the control, p = 0.0040 and 0.0683 respectively in the acute test **(B)** There were no significant changes in packed cell volume PCV (%) in papaya extract and no changes in the bitters group compared to the control, p = 0.9885 and 0.5353 respectively in the sub-chronic test, **(C)** There was a significant decrease in RBC levels in papaya extract (p = 0.0051) and no significant changes in bitters group (p = 0.0666) compared to the control, in the acute test **(D)** There were no significant changes in RBC levels in papaya extract (p = 0.3236) and bitters group (p = 0.4738) compared to the control, in the acute test as evaluated by Dunnett's post hoc test.

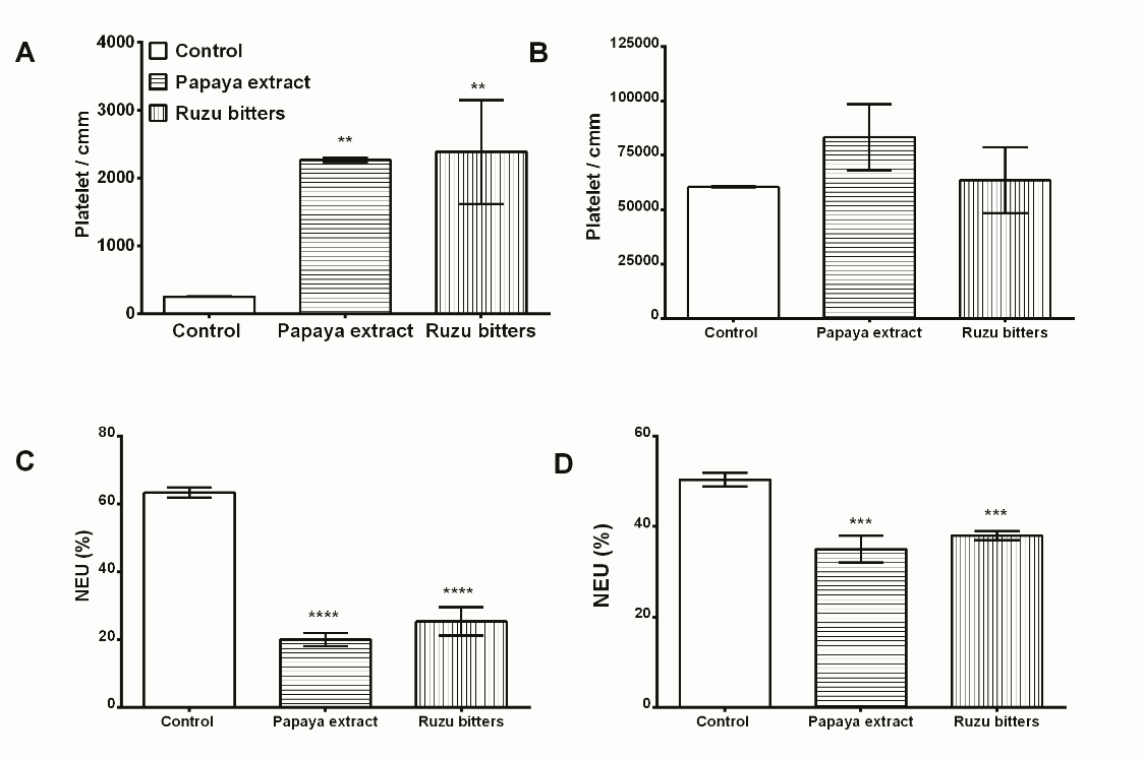

Figure 5 Mean platelet (/cmm) level and mean neutrophil (%) level of different groups in acute and chronic test respectively. Effect of extract and bitters on the mean platelet level (/cmm) and mean neutrophil (%) level in acute and sub-chronic administration. **(A)** There was a significant increase in mean platelet level in both papaya extract and bitters group compared to the control, p = 0.0025 and 0.0019 respectively in the acute test **(B)** There were no significant changes in mean platelet level (/cmm) in both papaya extract and bitters group compared to the control, p = 0.1101 and 0.9356 respectively in the sub-chronic test, **(C)** There was a significant decrease in mean neutrophil level in both papaya extract (p = 0.0001) and bitters group (p = 0.0001) compared to the control, in the acute test **(D)** There was a significant decrease in mean neutrophil level in both papaya extract (p = 0.0002) and bitters group (p = 0.0006) compared to the control, in the chronic test as evaluated by Dunnett's post hoc test.

The effect of extract and bitters on the mean lymphocyte level (%) and MCV (mean corpuscular volume) in the blood (FI) level in acute and sub-chronic administration revealed an increase in the levels of both parameters in the treatment groups compared to the control (Figure 6). Moreover, the effect of extract and bitters on the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in acute and sub-chronic administration revealed a significant increase in the MCH in both treatment groups compared to the control. As for the mean corpuscular hemoglobin concentration (MCHC), there were no significant changes in the acute test. However, in the sub-chronic test, there was a significant increase in MCHC for both treatment groups relative to the control (Figure 7).

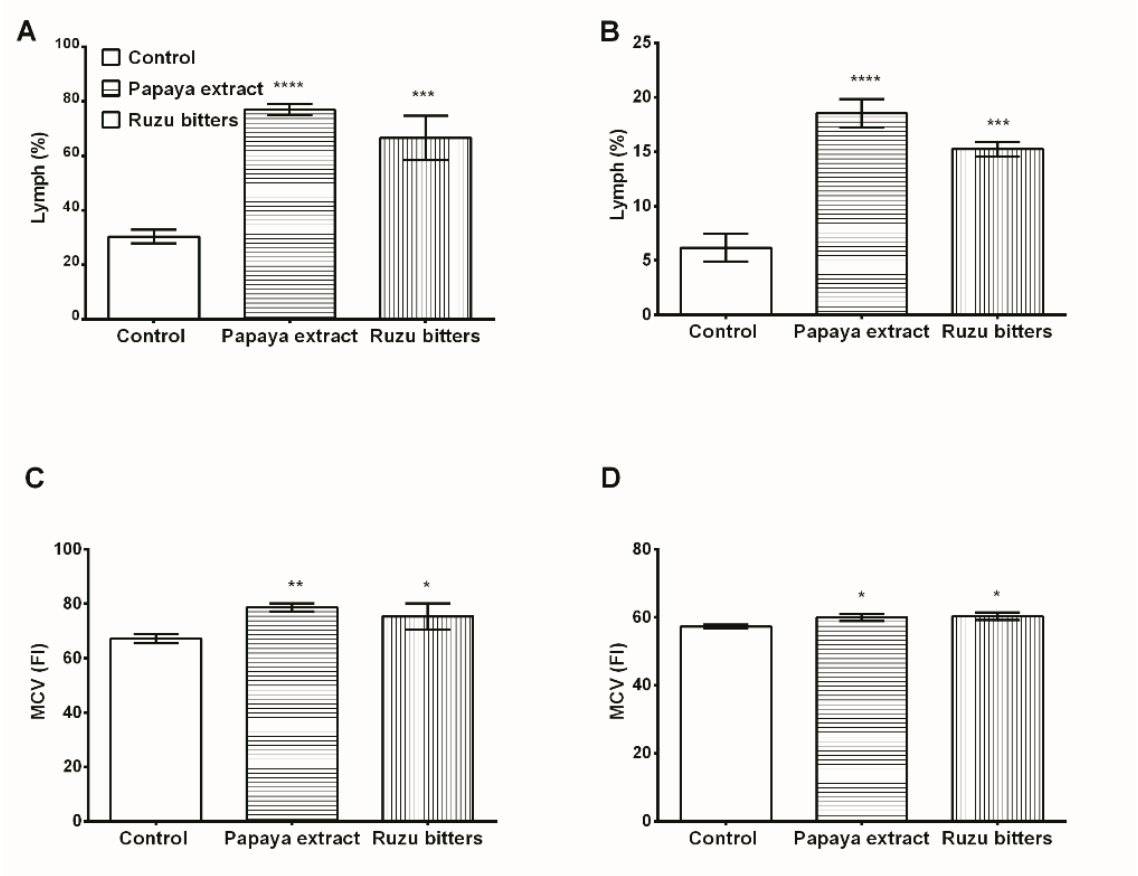

Figure 6 Mean lymphocyte level (%) and MCV (FI) level of different groups in acute and chronic test respectively. Effect of extract and bitters on the mean lymphocyte level (%) and MCV, mean corpuscular volume in the blood (FI) level in acute and sub-chronic administration. **(A)** There was a significant increase in mean lymphocyte level in both papaya extract and bitters group compared to the control, p = 0.0001 and 0.0002 respectively in the acute test **(B)** There was a significant increase in mean lymphocyte level in both papaya extract and bitters group compared to the control, p = 0.0001 and 0.0001 respectively in the sub-chronic test, **(C)** There was a significant increase in MCV level in both papaya extract (p = 0.0068) and bitters group (p = 0.0303) compared to the control, in the acute test **(D)** There was a significant increase in MCV level in both papaya extract (p = 0.0237) and bitters group (p = 0.0142) compared to the control, in the chronic test as evaluated by Dunnett's post hoc test.

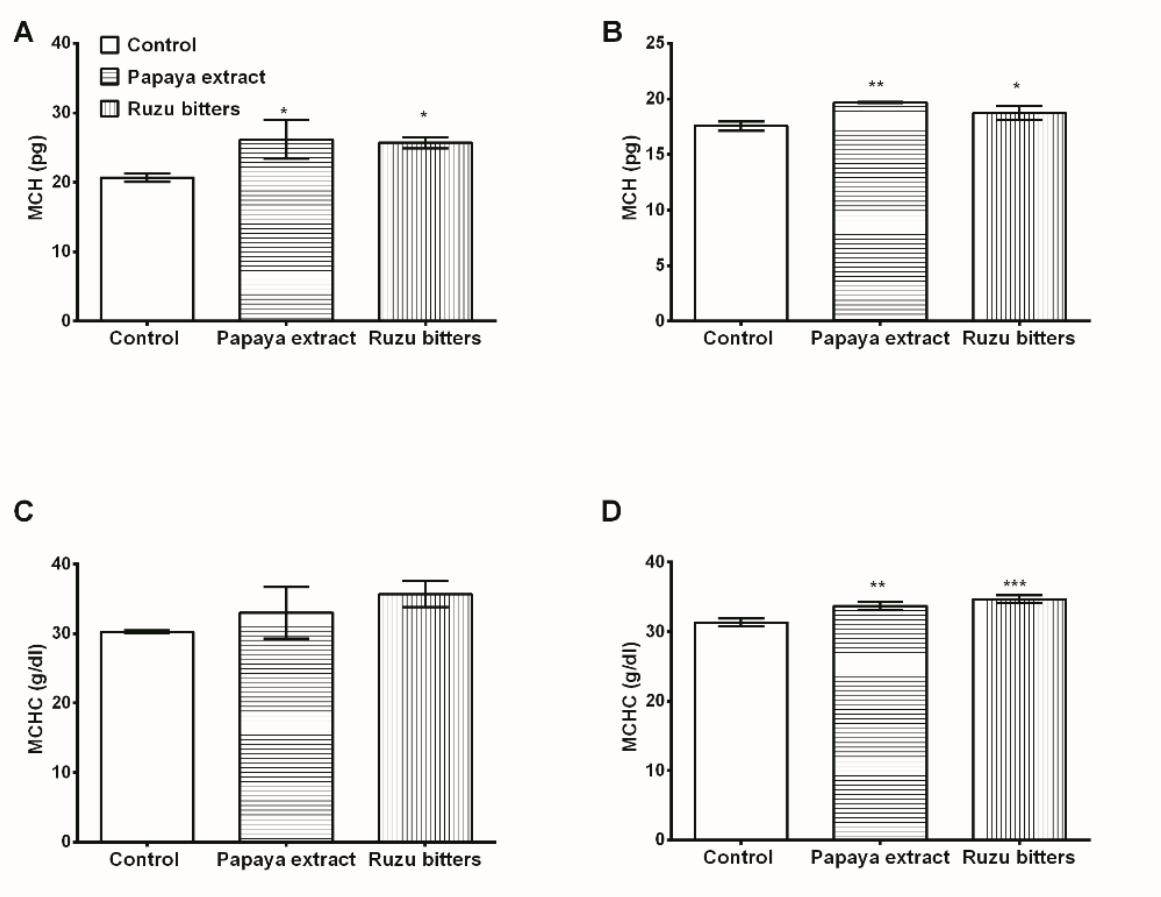

Figure 7 Mean corpuscular hemoglobin MCH (pg) and mean corpuscular hemoglobin concentration MCHC (g/dl) of different groups in acute and chronic test respectively. Effect of extract and bitters on the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in acute and sub-chronic administration. **(A)** There was a significant increase in MCH (pg) level in both papaya extract and bitters group compared to the control, p = 0.0129 and 0.0195 respectively in the acute test **(B)** There was a significant increase in MCH (pg) level in both papaya extract and bitters group compared to the control, p = 0.0021 and 0.0318 respectively in the sub-chronic test **(C)** There was no significant changes in MCHC level in both papaya extract (p = 0.3368) and bitters group (p = 0.0574) compared to the control, in the acute test **(D)** There was a significant increase in MCHC level in both papaya extract (p = 0.0046) and bitters group (p = 0.0007) compared to the control, in the chronic test as evaluated by Dunnett's post hoc test.

Effect of extract and bitters on the mean urea (mM^{-1}) and mean protein (g/L) level in acute and sub-chronic administration revealed no significant change in mean urea levels relative to the control, both in the acute and sub-chronic test. The Ruzu bitters group presented a significant increase in protein concentration in the acute test (Figure 8). Effect of extract and bitters on the mean ALT (U/L) and mean AST (U/L) levels in acute and sub-chronic administration revealed a significant increase in ALT. The Ruzu bitters group presented an increase in AST level (Figure 9). Effect of the extract and bitters on the mean ALP (U/L) and mean amylase (UI) levels in acute and sub-chronic administration revealed a significant decrease in ALP in both treatment groups, only in the acute test and not in the sub-chronic test (Figure 10).

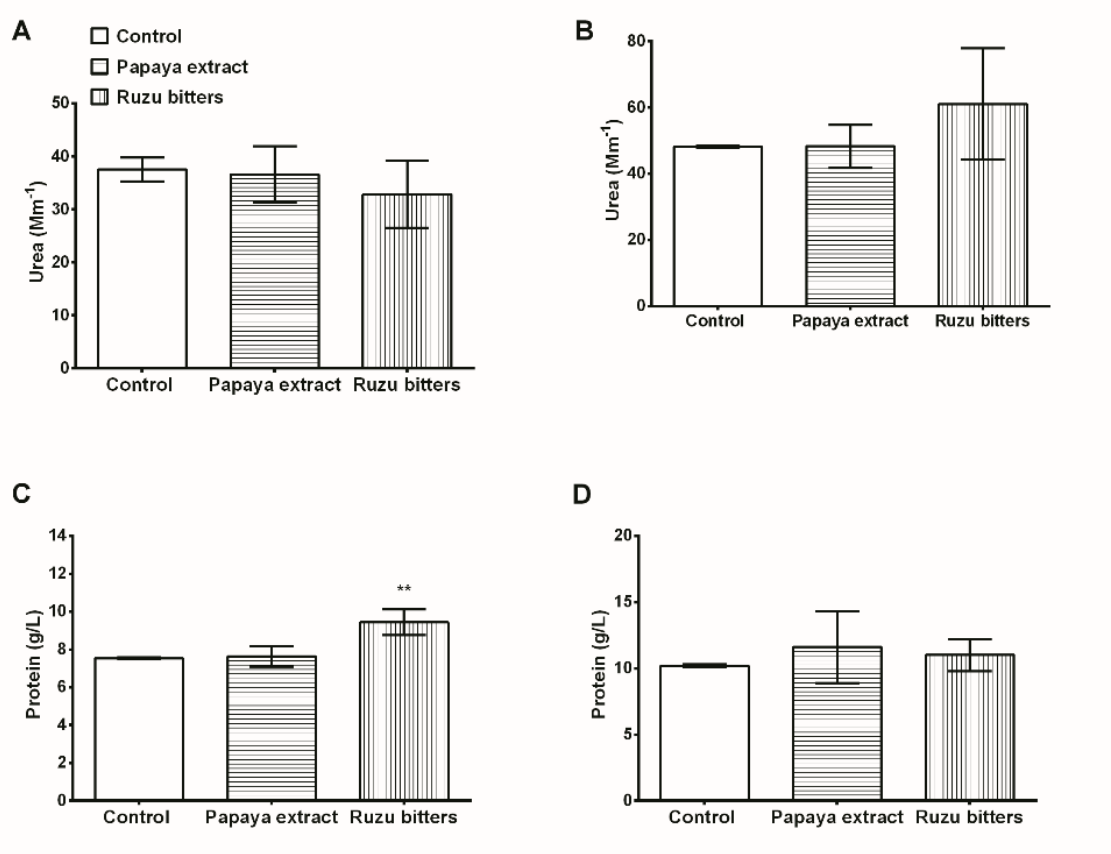

Figure 8 Mean urea (mM^{-1}) and mean protein (g/L) level of different groups in acute and chronic test respectively. Effect of extract and bitters on the mean urea (mM^{-1}) and mean protein (g/L) level in acute and sub-chronic administration. **(A)** There was no significant changes in mean urea (mM^{-1}) level in both papaya extract and bitters group compared to the control, p = 0.9621 and 0.4545 respectively in the acute test **(B)** There was no significant changes in mean urea (mM^{-1}) level in both papaya extract and bitters group compared to the control, p = 0.9999 and 0.2926 respectively in the sub-chronic test **(C)** There was no significant changes in mean protein (g/L) level in papaya extract (p = 0.9637), but a significant increase in mean protein (g/L) level in the bitters group (p = 0.0068) compared to the control, in the acute test **(D)** There was no significant changes in mean protein (g/L) level in both papaya extract (p = 0.5360) and bitters group (p = 0.7974) compared to the control, in the chronic test as evaluated by Dunnett's post hoc test.

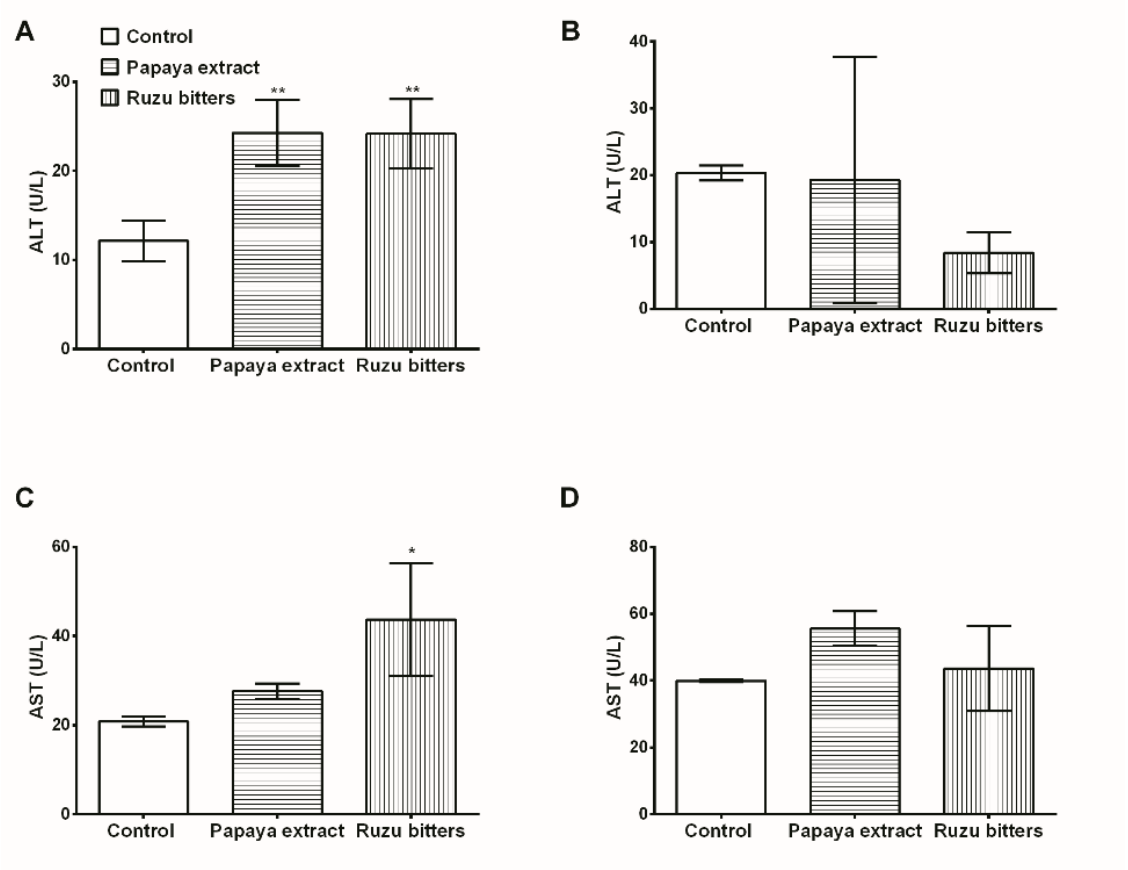

Figure 9 Mean alanine aminotransferase ALT (U/L) level and mean aspartate aminotransferase AST (U/L) level of different groups in acute and chronic test respectively. Effect of extract and bitters on the mean ALT (U/L) and mean AST (U/L) level in acute and sub-chronic administration. **(A)** There was a significant increase in mean ALT (U/L) level in both papaya extract and bitters group compared to the control, $p = 0.0079$ and 0.0082 respectively in the acute test **(B)** There was no significant changes in mean ALT (U/L) level in both papaya extract and bitters group compared to the control, $p = 0.9899$ and 0.3562 respectively in the sub-chronic test **(C)** There was no significant changes in mean AST (U/L) level in papaya extract ($p = 0.4688$), but a significant increase in mean AST (U/L) level in the bitters group ($p = 0.0163$) compared to the control, in the acute test **(D)** There was no significant changes in mean AST (U/L) level in both papaya extract ($p = 0.0886$) and bitters group ($p = 0.8043$) compared to the control, in the chronic test as evaluated by Dunnett's post hoc test.

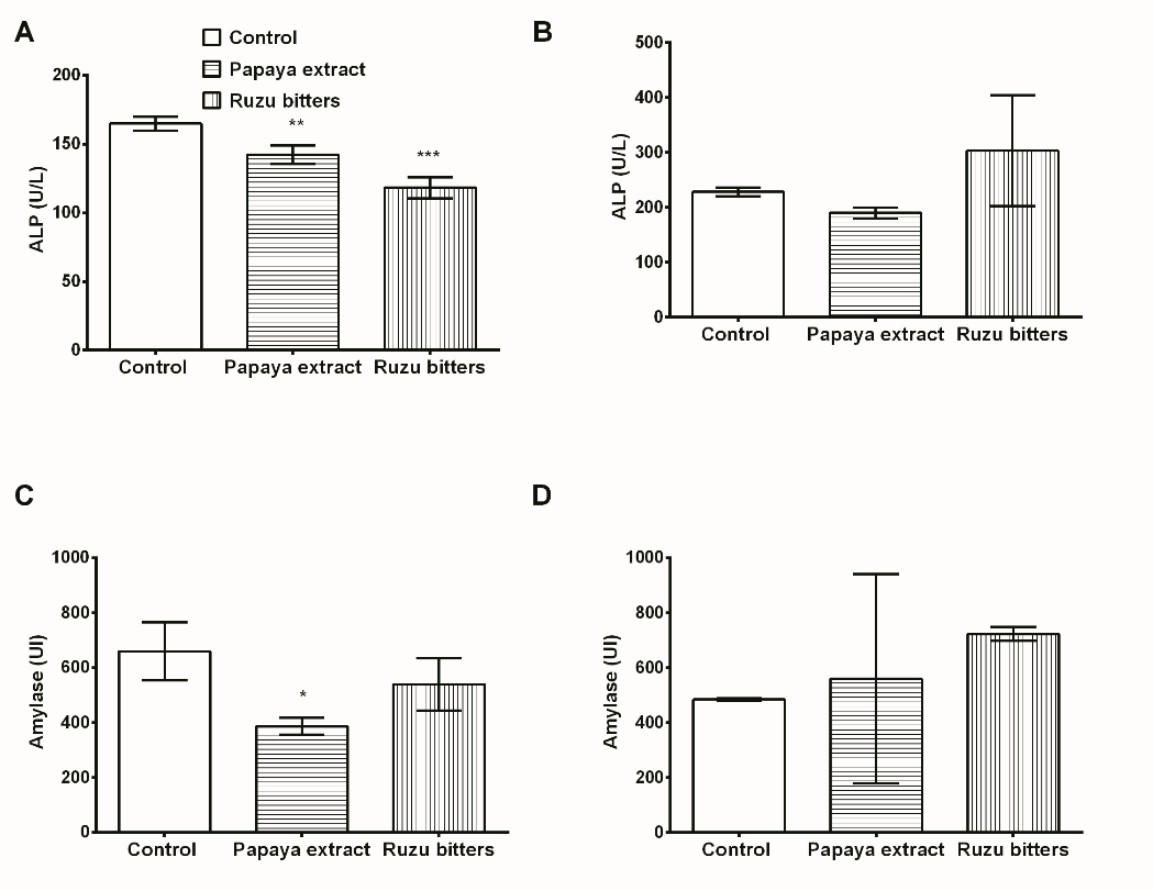

Figure 10 Mean alkaline phosphatase ALP (U/L) level and mean amylase (UI) level of different groups in acute and chronic test respectively. Effect of extract and bitters on the mean ALP (U/L) and mean amylase (UI) level in acute and sub-chronic administration. **(A)** There was a significant decrease in mean ALP (U/L) level in both papaya extract and bitters group compared to the control, p = 0.0099 and 0.0003 respectively in the acute test **(B)** There was no significant changes in mean ALP (U/L) level in both papaya extract and bitters group compared to the control, p = 0.6600 and 0.2726 respectively in the sub-chronic test **(C)** There was a significant decrease in mean amylase (UI) level in papaya extract (p = 0.0130), but no significant change in mean amylase (UI) level in the bitters group (p = 0.2171) compared to the control, in the acute test **(D)** There was no significant changes in mean amylase (UI) level in both papaya extract (p = 0.8853) and bitters group (p = 0.3722) compared to the control, in the chronic test as evaluated by Dunnett's post hoc test.

Antioxidant activities in both acute and sub-chronic administration revealed a significant decrease in antioxidant activities of enzymes GSH, SOD and CAT in both treatment groups relative to the control. However, MDA was significantly decreased in the acute test, for both treatment groups relative to the control (Table 1 and Table 2).

Table 1 Antioxidant activity in the control, extract and Ruzu bitters groups after the acute test (7 days).

Antioxidants	Control	*C. p.* leaves extract	Ruzu bitters
GSH (U/mg pro)	20.51 ± 0.04	14.58 ± 0.09[a]	16.82 ± 0.47[b]
SOD (U/mg pro)	5.65 ± 0.01	4.21 ± 0.09[c]	3.81 ± 0.47[b]
CAT (U/mg pro)	22.38 ± 1.31	20.63 ± 0.94	14.81 ± 0.82[b]
MDA (U/mg pro)	3.15 ± 0.02	1.47 ± 0.15[a]	0.75 ± 0.16[a]

Table 2 Antioxidant activity in the control, extract and Ruzu bitters groups after the sub-chronic test (14 days).

Antioxidants	Control	*C. p.* leaves extract	Ruzu bitters
GSH (U/mg pro)	27.41 ± 0.47	20.32 ± 0.47[a]	24.85 ± 0.89[c]
SOD (U/mg pro)	4.51 ± 0.48	4.53 ± 0.13	2.86 ± 0.09[b]
CAT (U/mg pro)	617.19 ± 0.95	543.91 ± 0.42[a]	543.98 ± 1.49[a]
MDA (U/mg pro)	0.32 ± 0.08	1.65 ± 0.01[c]	4.48 ± 0.48[a]

5. Discussion

Most fruits and vegetables are rich in antioxidant phytoconstituents. Phytochemicals are free radical scavenging agents. Hence, they possess health-promoting and disease-prevention properties [27]. Most of them can do these, chiefly due to the synergistic actions of their bio-active components [28]. For instance, Tetraena simplex (T. simplex), a newly identified specie of the family Zygophyllaceae was reported to possess both antioxidant and antibacterial activities [29]. The methanolic extract of Zingiber officinale (Roscoe) rhizome (MEZOR), was also reported to ameliorate the onset of chemically-induced liver damage [30].

Over the years, herbal bitters have increased usage and popularity (detox herbal cocktail mixtures). Most of them stimulate digestive secretions and metabolism by increasing appetite. Thus, they relieve constipation and aid digestion. However, despite the previously reported non-toxic phenotypic measures, a little scientific basis exists for the efficacy and safety of the constituents of Ruzu bitters and *Carica papaya* leaves extract. Ruzu bitters is a newly introduced herbal bitter into our community; its safety consumption should be established. To ascertain the possible toxicity effects of Ruzu bitters and *Carica papaya* leaves extract, their effect on biochemical parameters, brain histological and hematology of treated rats were evaluated. Experimental animals were administered the two supplements at acute and sub-chronic levels. Brain sections and blood serum samples were assayed for biochemical parameters.

The effect of the extracts on body weight revealed that rats administered the Ruzu herbal bitters exhibited a gradual loss in weight. However, those administered *Carica papaya* leaves extract experienced a gradual increase in body weight relative to the control and the Ruzu bitters group (Figure 1). This observation is not surprising, the Ruzu bitters is a strong laxative *i.e.*, it promotes bowel movements. People who consume certain bitters tend to eat less and lose weight with time [14]. The increase in body weight exhibited by the *Carica papaya* leaves extract group could be

attributed to its nutritive properties. The leaves contain phytonutrients, vitamins and minerals essential for easily absorbing other nutrients. The leaf extract was equally reported to have gastric protection properties [17] and an anti-diabetic effect [31]. Also, the leaves extract possesses anti-cancer properties; black papaya seeds exert a protective effect on prostate cancer cell formation [10, 32]. However, *Carica papaya* was reported to induce weight loss [33].

The leaves have been used as a nutraceutical containing carbohydrates, minerals, vitamins, lipids and proteins. Therefore, the nutritive and medicinal properties are well documented. The composition in varying proportions includes; carbohydrates 8.3%, ascorbic acid 38.6%, protein 5.6%, minerals such as magnesium 0.035%, iron 0.0064% and phosphoric acid 0.225% per 100 grams of edible serving [7].

The brain histopathology report of the present study revealed aggregates of cells with large pleomorphic hyper-chromatic nuclei, not disposed of as ducts, but as sheets. There appeared to be atypia (structural abnormalities) in the brain of rats administered Ruzu bitters after 14 days compared to the control, and papaya extract group. However, the brain tissue of the rats administered papaya leaves was as intact as those of the control group, which was not the case in the Ruzu bitters group (Figure 2). This could result from the antioxidative protective effects of the phytoconstituents in the extract of *Carica papaya* with their synergistic activities. We hereby reveal *Carica papaya* leaves extract as a neuroprotective agent. The leaf extract has been reported to have a protective effect against gamma-radiation-induced oxidative damage [23]. Ruzu bitters, on the other hand, does not consist of *Carica papaya* as part of its components. Perhaps, some food processing companies may henceforth consider *Carica papaya* leaves extract as part of its herbal component.

The hematological effects of papaya extract and Ruzu bitters were carefully evaluated at the acute and chronic levels. The white blood cell is a very important component of the human health condition of individuals; if abnormally present, it could be detrimental to human health, by causing different diseases. In the acute test, the study showed an elevated white blood cell count in rats administered papaya extract, compared to Ruzu bitters and control rats (Figure 3). This is in line with previous studies which stated that papaya leaves extract exhibits antitumor activity via its immunomodulatory properties [34, 35]; another study equally supported its health-promoting and immunoprotective effects [36].

Mean PCV (%) and mean red blood cell RBC ($\times 10^{12}$/L) levels of different treated groups in the acute and chronic tests, respectively, revealed significantly low levels in the papaya group compared to the other groups ($p < 0.0040$, Figure 4). This could suggest a possible mild hemolytic effect of *C. papaya* leaves extract. This is in line with previous studies which revealed the hemolytic effect of the papaya leaf extract [37]. However, this may not necessarily lead to anemia, due to the impressive effect of the extract in enhancing an increased level of the MCV, MCH and MCHC, in a both acute and chronic tests done for the treatment groups compared to the control. The mean lymphocyte level in papaya extract and Ruzu bitters groups, for both acute and chronic groups, increased significantly compared to the control group, which had lower lymphocytes (Figure 6 and Figure 7).

The mean neutrophil level in the papaya extract and Ruzu bitters group decreased significantly compared to the control group in the test for acute and chronic levels. Neutrophils level showed a significant increase (Figure 5) in control rats compared with experiment rats. A neutrophil is a type of white blood cell that responds to bacterial infection, and high levels could be from some

inflammation. Therefore, these reported low levels might be that papaya leaf extract has anti-inflammatory and immunomodulatory effects. Platelet levels are significantly higher ($p < 0.05$) in the papaya extract group of both acute and chronic tests compared to those of Ruzu and the control group (Figure 5). This result is by a recent study which shows that platelet counts significantly increased in patients with dengue fever and dengue hemorrhagic fever treated with *Carica papaya* leaves extract [18] ; this is a result of its immunomodulatory effect [38], as reported in another study which shows that it was able to mediate allergic reactions and inhibit the growth of certain tumor cells, the leaves contains flavonoids, a potent antioxidant, alpha-tocopherol, and benzyl isothiocyanate, which increases the production of Th1-type cytokines [39]. Another study revealed that the leaf extract showed a significant therapeutic effect on Thrombocytopenia, a clinical manifestation of low platelet count [40]; this is also in line with our result.

MCV, MCH and MCHC are the three main red blood cell indices that help measure the red blood cells average size and hemoglobin composition. In this study, they were significantly higher. MCV, MCH and MCHC for both the acute and chronic tests done for the treatment groups compared to the control (Figure 7). The mild hemolytic response earlier stated in the *Carica papaya* group in this study did not necessarily lead to anemia.

The results obtained in this study showed that the extract of *C. papaya* leaves caused non-significant changes in urea and protein levels. As for the amylase test, the control group had the highest level, but there were no significant changes in the levels of amylase in both acute and chronic tests for the Ruzu bitters compared to the control. There was a significant decrease in the amylase levels in the acute test in the papaya group (Figure 8).

Equally, there were no significant changes ($p > 0.05$) in the levels of ALT, AST and ALP (Figure 9-10). Any elevation in the levels of ALP and ALT above the normal ranges (that of the control) could be associated with cell necrosis of many tissues in the body, such as myocardial infarction, liver disorder, toxic jaundice, and the like.

As for the antioxidant activities, the GSH activities for papaya extract and Ruzu bitters in the acute test revealed a significantly higher activity than the control group; $p < 0.0001$ for both test groups. (Table 1 and Table 2). GSH activity in the chronic administration revealed a significantly higher effect than the control group, $p < 0.0001$ and 0.0049 for the papaya and Ruzu groups respectively. There was a significant increase in SOD activity in the acute test in the papaya ($p < 0.0012$) and the Ruzu ($p < 0.0003$) groups, respectively. As for the chronic test, there were no significant changes in the SOD activity of the papaya extract ($p = 0.9948$). There was a significant increase in the SOD activity of the Ruzu bitters ($p = 0.0008$) compared to the control group. As for the CAT activity (acute test), there were no significant changes in the activity ($p = 0.1451$) in the papaya group, there was a significant increase in CAT activity in the Ruzu bitters group ($p = 0.0002$) compared to the control group. As for the CAT activity (chronic test), there was a significant change in the activity of both the papaya and Ruzu bitters group ($p < 0.0001$) compared to the control. The aqueous extract of *Carica papaya* is less cytotoxic in C6/36 cells compared with its fractions which showed better antioxidant activity. The leaf stores potent antioxidants capable of scavenging free radicals [41].

To ascertain the effect of the extract on oxidative stress modulation, a marker of oxidative stress, MDA; malondialdehyde was measured, and there were significantly lowered MDA levels in the acute administration of papaya and Ruzu bitters ($p < 0.0001$). However, the chronic administration caused a significant increase in MDA levels in both test groups ($p = 0.0021$) and ($p < 0.0001$) for both papaya and Ruzu bitters respectively. This could suggest mild oxidative stress in the chronic

administration of both treatments. However, it was reported that *Carica papaya* extract does not exhibit an adverse effect on acute and chronic administration in the safety and oral toxicity administration of the leaf extract [42]. Hence, it is a safe potential source of pharmaceutical products for therapeutic use [43].

6. Conclusion

Long-term administration of ethanol leaf extract of *Carica papaya* in rats resulted in weight gain with no adverse effect on liver function enzymes; hence, it is non-toxic to the liver with a good immunological effect as shown in the present study. *Carica papaya* extract decreases neutrophil levels, which reveals a possible anti-inflammatory effect. Increased antioxidant activity and decreased oxidative stress markers such as MDA, ALT and ALP suggest anti-oxidative stress activity. Nevertheless, the present findings have shown that Ruzu Herbal bitters cause a reduction in body weight and altered brain structure with increased neutrophils, ALT and AST levels; however, it is not likely to produce severe toxicological effects on other hematological and biochemical indices measured in this study, when given at normal therapeutic doses. *Carica papaya* leaf extract has immunomodulatory, anti-inflammatory, and anti-hemolytic effects. Further research on the long-term administration effect of both extracts on other vital organs is recommended. Regular use of herbal bitters or plant extracts should only be under strict medical supervision, while the constant habitual use calls for caution.

List of Abbreviations and Units of Measurement

ALT: Alanine amino transaminase, **AST**: Aspartate amino transaminase, **WBC**: White blood cell, **HGB**: Hemoglobin, **PCV**: Part cell volume, **RBC**: Red blood cell, M**CV**: Mean corpuscular volume, **MCH**: Mean Corpuscular hemoglobin, **MCHC**: Mean corpuscular hemoglobin concentration. (g/L): gram per liter, (U/L): Unit per liter, (mM^{-1}): per millimolar.

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Author Contributions

Professor O. A. T. Ebuehi worked on the project development and supervision, Mr. Lasekan Ayobami worked on data collection and analysis, while Dr. Ajagun-Ogunleye worked on data analysis, result presentation and writing. All the authors contributed to the write-up of the manuscript.

Competing Interests

The authors declare that they have no competing interests.

Availability of Data and Materials

The data presented in this manuscript are part of the academic research of Mr. Lasekan Ayobami. The data has not been deposited in any repository, however, it will be made available to researchers upon request.

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