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Histo-biochemical analyses of the ameliorative potentials of *Phyllanthus nuriri* and *Carica papaya* on some visceral of Albino rats previously treated with Nitrofuratoin

Omorodion, N.T. and Nwibana Barisuka Kiofi[2]

[1]Department of Medical Laboratory Sciences,
School of Basic Medical Sciences,
College of Medicine
University of Benin,
Benin City, Nigeria

River State College of Health Sciences and Management Technology,
Portharcourt,
River State, Nigeria

Email: terry.omorodion@uniben.edu

Abstract
Herbal medicine has been used since the days of our forefathers. Nitrofuratoin is a synthetic drug used in the treatment of bacterial infections. This study was aimed at the ameliorative potential of Phyllanthus nuriri and Carica papaya on liver, kidney and lungs histomorphologies of albino rats pre-treated With nitrofuratoin. Twenty eight (28) rats were used for this research work. The research was in two stages. The animals were in five groups in stage one, group 1 with 8 animals received rat pellet and water and was used as control while group 2, 3, 4, and 5 with five rats each received 100 mg/kg body weight of nitrofuratoin. After 14 days of administration, four rats from group the control group (1) and four rats from the test groups were sacrificed, tissues were harvested taken for histological evaluation. In stage two, group 1and 2, received rat pellet and water and served as positive and negative control, group 3, 4,and 5 received 100mg/kgbody weight of Phyllanthus nuriri, Carica papaya and combine extract respectively. After 14 days of administration, Animals used as control experiment and test were weighed, sacrificed via cranial dislocation, and tissues harvested for histopathological evaluation There were significant increase ($P < 0.05$) in potassium, chloride and bicarbonate and also deleterious effect on the kidney, liver and lungs in nitrofuratoin administered rats. All these physiological and morphological alterations were repaired with P. niruri and C. papaya and combined plant extract. The plant extract could serve as cheap and affordable treatment for kidney, lungs and liver inflammation.

Keywords: *Phyllanthus niruri, Carica papaya,*

INTRODUCTION
As a result of its widespread use in traditional medicine, particularly in Asia, South America, and Africa, *Phyllanthus niruri* is well-known throughout the world. It belongs to the plant family Euphorbiaceae, which also includes shrubs and herbs with milky juice that are upright or prostrate. Numerous preclinical and clinical studies have shown the value of *P. niruri*

*Author for Correspondence

extracts, and they are well known to have a range of therapeutic effects (Patel *et al*., 2011). Brazilian researchers looked into a *P. niruri* alkaloid's antispasmodic properties (Patel *et al*., 2011).

Due to its anti-hepatitis B activities *P. niruri* attracted attention on a global scale in the late 1980s (Bagalkotkar et al., 2006).

The Caricaceae family includes the *Carica papaya*. It is cultivated and grown all over tropical America, where it is native. Papain and chymopapain are examples of the proteolytic enzymes found in *C. papaya*. Alkaloid carpine is present in the seeds, leaves, and unripe fruits in trace amounts (Orwa *et al*., 2009). Many people use C. papaya's medicinal qualities. Chewing the root and consuming the juice is used to treat bronchitis and other respiratory illnesses (Ayoola *et al*., 2010; Atta, 1999). The dried, brown pawpaw leaf is the best tonic and blood purifier, while the fresh, green pawpaw leaf is antiseptic (Atta, 1999), It functions as an antimalarial and an antisickling agent in sickle cell anemia. The leaf is also used to treat liver conditions and dengue fever. Nasal congestion can also be relieved by chewing the seeds of ripe pawpaw fruit (Atta, 1999). The green, unripe pawpaw is said to have therapeutic value due to its antiseptic qualities (Ayoola *et al*., 2010). The green papaya leaf tea aids in the treatment of diseases like arteriosclerosis, high blood pressure, chronic indigestion, obesity, and overweight as well as the promotion of digestion (Ayoola *et al*., 2010).

Since 25 years ago, nitrofurantoin, also known as 1-[(5-Nitro-2-furanyl) methylideneamino] imidazolidine-2,4-dione. Nitrofuratoin has been used extensively to treat bacterial urinary tract infections. It achieves effective antibacterial concentrations in the urine due to its quick absorption following oral administration, minimal biotransformation, high solubility, and excretion in the urine (Cohen, S.M., 1978). Nitrofuratoin is particularly effective for this indication because susceptible bacteria cannot develop resistance (Cohen, 1978). Simple urinary tract infections are treated or prevented with nitrofurantoin. It is now regarded as a second-line treatment for this kind of infection due to the emergence of bacterial resistance (Stamm et al.,1993). The bioactivation of nitrofurantoin results in a number of reactive intermediates that disrupt bacterial ribosomal proteins and prevent the synthesis of critical cellular macromolecules, such as DNA (Guay, 2001). With a half-life of roughly 0.3–1 hours, nitrofurantoin is quickly absorbed by the gastrointestinal tract, quickly excreted by the kidneys, and concentrated in the urine (Conklin, 1978). Adults with normal renal function (creatinine clearance > 60 mL/min) should take 50–100 mg via oral route once every day for a week as a treatment or 50–100 mg via oral route at bedtime as a preventative measure (Petri, 2004). Nitrofurantoin's bactericidal effects are diminished in patients with impaired creatinine clearance because the drug's clearance is governed by glomerular function (Oplinger, M., Andrews, C.O (2013).

The indiscriminate use of synthetic drugs in our society today is alarming and with the increasing economic sham, not everyone can afford the drugs. This issue of over-the-counter usage is worrisome and need to be tackled. The easier means of tackling this menace could be the use of herbal medicine, which is less toxic and effective due to its freshness and being taken from the source. There is no monitoring or control in place to curtain the use of the herbal drugs also the dosage need to be worked upon to avoid the damaging effect that can result from its usage.

Nitrofuratoin is a drug that is being used in the treatment of bacterial infection (Aremu, 2021). Nigerians use it indiscriminately because there are no measures in place to control its usage.

The aforementioned, reasons necessitated this research. Hence, the research study is aimed at ascertaining ameliorative potential of *Phyllanthus nuriri* and *Carica papaya* on some histomorphologies of albino rats pre- treated with nitrofurantoin.

MATERIALS AND METHOD

Ethical Consideration

The National Research Council's Guidance for the Care, Handling, and Use of Laboratory Animals was adhered to when conducting the study (NRC, 2012).

Collection of samples

The entire plant of *Phyllanthus niruri* and *Carica papaya* including the stem, root, and leaves were collected from the garden of University of Benin and thereafter taken to Mr. H. Akinbosun in the Department of Botany, University of Benin for identification and authentication.

Laboratory procedures

Water was used to wash the entire *Phyllanthus niruri and Carica papaya* plant. They were divided into small pieces, air dried in shade for a few days, and then further dried for three days at 45^{o}C before being ground into powder and kept in an airtight container.
The powder was extracted with ethanol and water (3:1) on a reflex water bath for 3 hours. The cycle was repeated three times. The extract was concentrated on a rotary flash evaporator and air-dried to produce the semisolid extract.

Analytical Phytochemistry

phytochemical components in the blended powder of the seeds, a qualitative phytochemical analysis was carried out using a technique described by Owoyele *et al*., (2011)

Acute Toxicity Study

The acute toxicity study was conducted using Lorke's methodology. The formula LD50 = (Highest nonlethal dose) x was used to calculate the median lethal dose (LD50) (Lowest lethal dose). The maximum dosage that an animal could be exposed to accidentally served as the basis for the experiment's dosage.

Animal Acclimatization

In this experiment, a total of twenty eight rats (28) Animals were housed in polyvinyl cages under typical laboratory conditions, including temperature (25^{0}C, 10^{O}C) light cycle, and food and water availability (12h light and 12h dark). They were given pelletized food, and clean water was available at all times. Male and female rats were housed in separate cages from their female counterparts both before and during the study period to prevent conception. Before the experiment, the animals were given a two-week acclimatization period.

Grouping and Administration

The study was in two stages. Stage one was to check for toxicity of nitrofuratoin, while the second stage was to see the ameliorative effect of the two extracts on the tissue cells and organs. In stage one, the rats were divided into two, 8 rats in group 1 and five rats each in group 2, 3, 4 and 5.

Stage 1:
GROUP 1: Rat pellets, water, and lithium were given to the control group.

GROUP 2, 3, 4, and 5: Nitrofuratoin 100 mg/kg body weight was administered.

Four rats from group 1 and four from the test groups were randomly selected and sacrificed via cranial dislocation after receiving the reference drug every day for 14 days. The blood was drawn from the heart for a complete blood count, and the kidney, liver, and lung tissues were collected for histopathological analysis.

Stage 2:
Group 2 received rat pellets, water *ad lithium* as a control (previously treated with nitrofuratoin)

GROUP 3: *Phyllanthus nuriri* 100 mg/kg body weight was administered.

GROUP 4: Received *Carica papaya* at a dose of 100 mg/kg body weight.

GROUP 5: Received 100 mg/kg body weight equal proportion of *Carica papaya and Phyllanthus niruri*.
After 14 days of administration, all the animals in group one, two three and four were taken from the cage and sacrificed via cranial dislocation, the blood samples taken from the heart for complete blood count, the kidney, liver and lung tissues harvested for histopathological evaluation

Biochemical Evaluation
Blood samples were obtained for the estimation of two kidney function indicators and two liver enzymes, aspartate aminotransferase , alanine aminotransferase, Creatinine and Urea. This analysis was conducted using a chemistry auto-analyzer.

Haematoxylin and Eosin Staining
The protocol was carried out using procedure described by Omorodion *et al*., (2022).

Statistical analysis
One-way ANOVA and Turkeys multiple comparison tests were used in GraphPad Prism 8 to compare the treatment groups of Phyllanthus niruri and Carica papaya to the control group (GraphPad Software, Inc., CA, USA). With a p-value of 0.05 or less, differences were considered statistically significant.

RESULTS
In this study, we found out that the kidney markers potassium, chloride, and bicarbonate significantly increased (P 0.05) in the animals given nitrofuratoin, but the parameters decreased after the administration, as was the case in the untreated group (previously given nitrofuratoin), compared to the control (fig. 1, 2 and 6 above). Potassium and chloride values in the groups given *C. papaya, P. niruri*, and combined extract did not differ significantly (P > 0.05) (previously treated with nitrofuratoin). The values of sodium, creatinine, and urea in the nitrofuratoin group compared to the control group did not differ significantly (P > 0.05). (fig. 3, 4, 5), The value of sodium (Na+) significantly increased (P 0.05) in the untreated group as well as in the groups treated with *C. papaya, P. niruri,* and combine extract. There was no significant difference (P > 0.05) in the value of urea in the groups treated with *C. papaya* and combine, only a significant increase (P 0.05) in the value of urea in the group treated with *P. niruri*.

According to our findings, there was a significant increase in the value of alkaline phosphatase (ALP) enzyme in the group treated with nitrofuratoin, a marginal increase in the untreated group, a significant increase (P 0.05) in the group treated with *C. papaya*, but only marginal differences in the groups treated with P. niruri and combine extract (P > 0.05). (fig 7). All groups' values for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin did not differ significantly (P > 0.05). (figure 8, 9 and10)

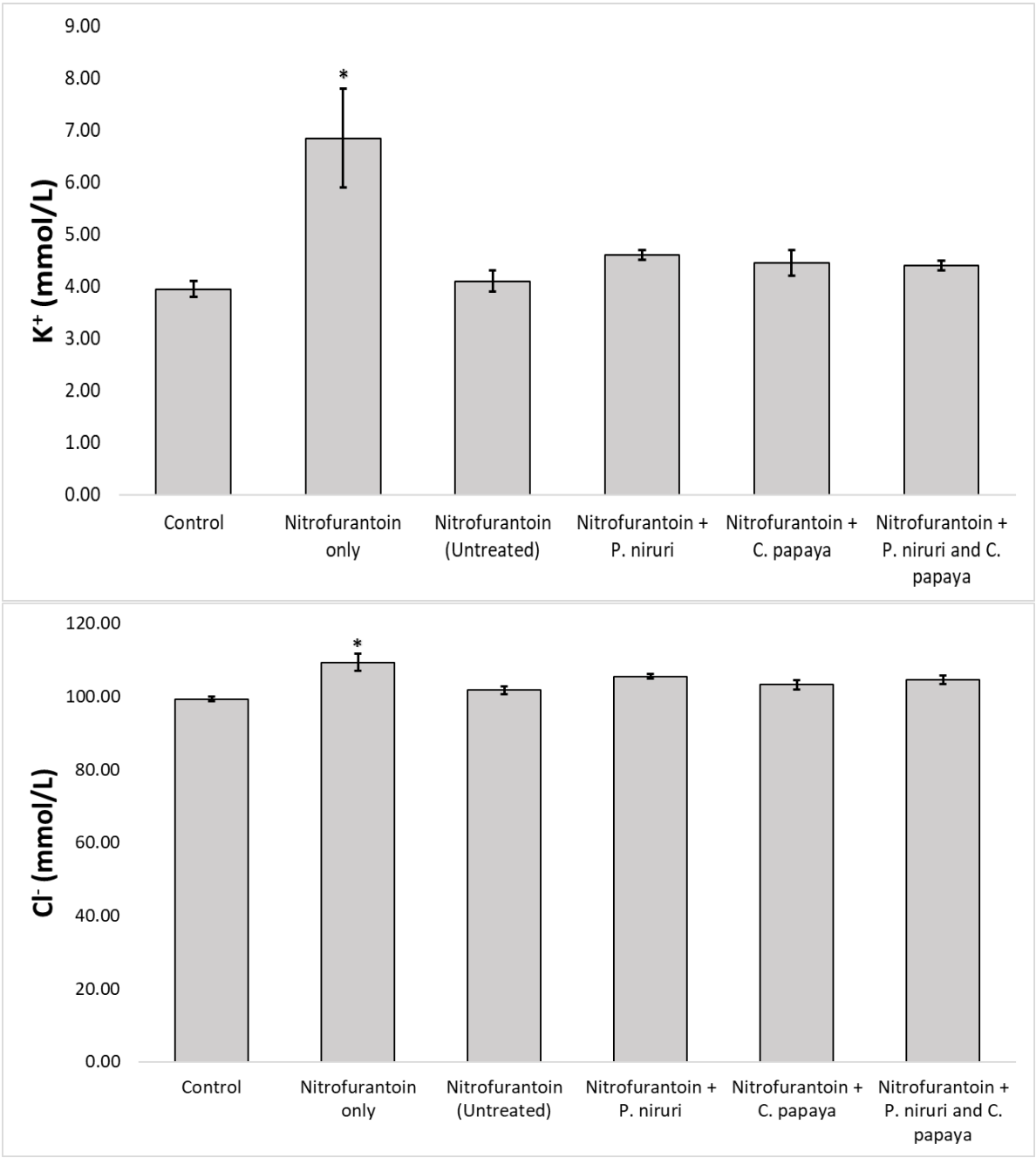

Figure 1: Chart showing the levels of potassium and chloride in both test and control
Significant increase (P<0.05) were observed in the value of potassium in Group B, when compared to the control group, There was a statistically significant increase (P<0.05) of chloride in Group B, when compared to the control group.
*significantly different from the control group

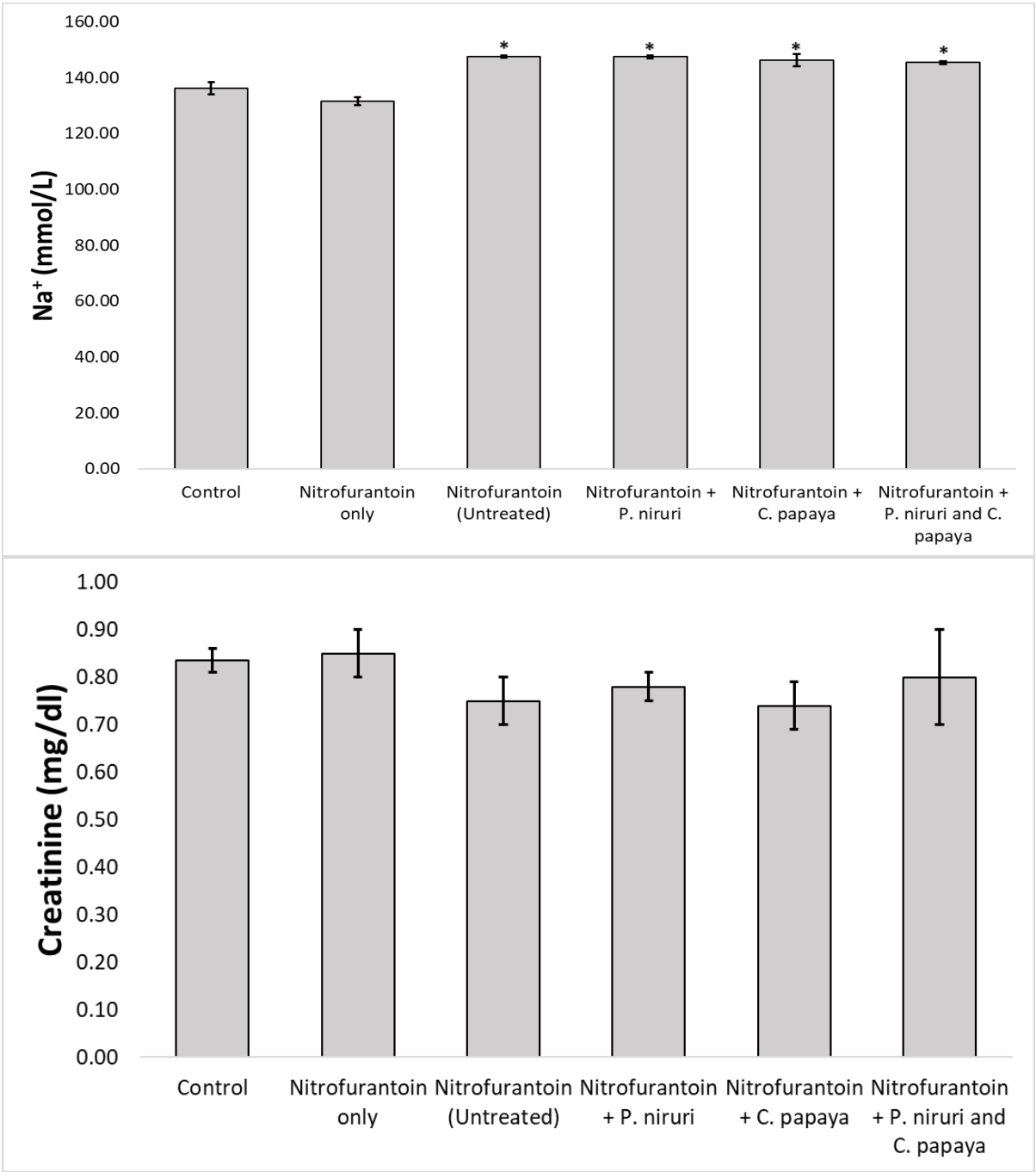

Figure 2: Chart showing the levels of sodium and creatinine in test and control groups
*significantly different from the control group
Significant increases ($P<0.05$) observed in the value of sodium in Groups C, D, E, and F, when compared to the control group, There were no statistically significant differences ($P>0.05$) in the levels of creatinine across the groups

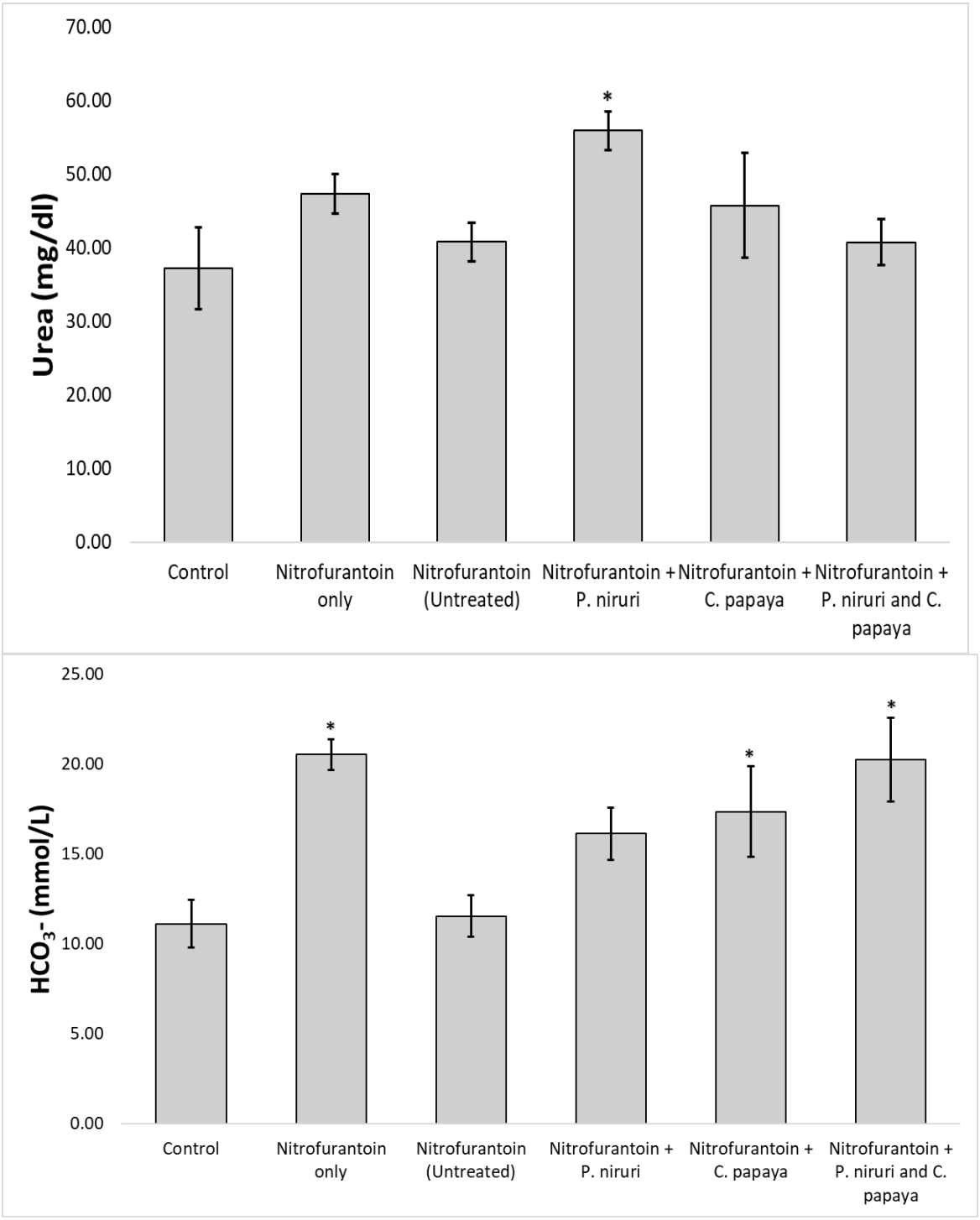

Figure 3: Chart showing the levels of urea and bicarbonate
Significant increase ($P<0.05$) observed in the value of urea in Group D, when compared to the control group, There were statistically significant increases ($P<0.05$) of bicarbonate in Groups B, E, and F, when compared to the control group.
*significantly different from the control group

Liver function tests

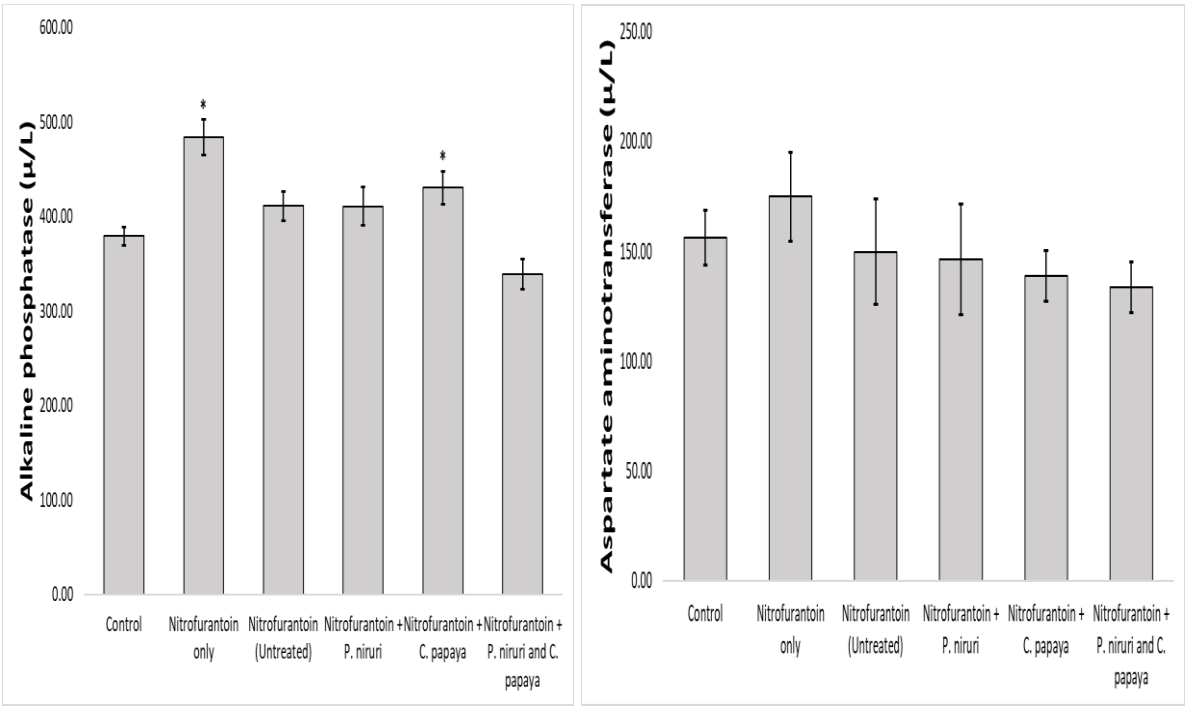

Figure 4: Chart showing levels of alkaline phosphatase and aspartate aminotransferase
*significantly different from the control group
Statistically significant increases (P<0.05) observed in the value of ALP in Groups B and E, when compared to the control group, there were no statistically significant differences (P>0.05) of aspartate aminotransferase across the groups.

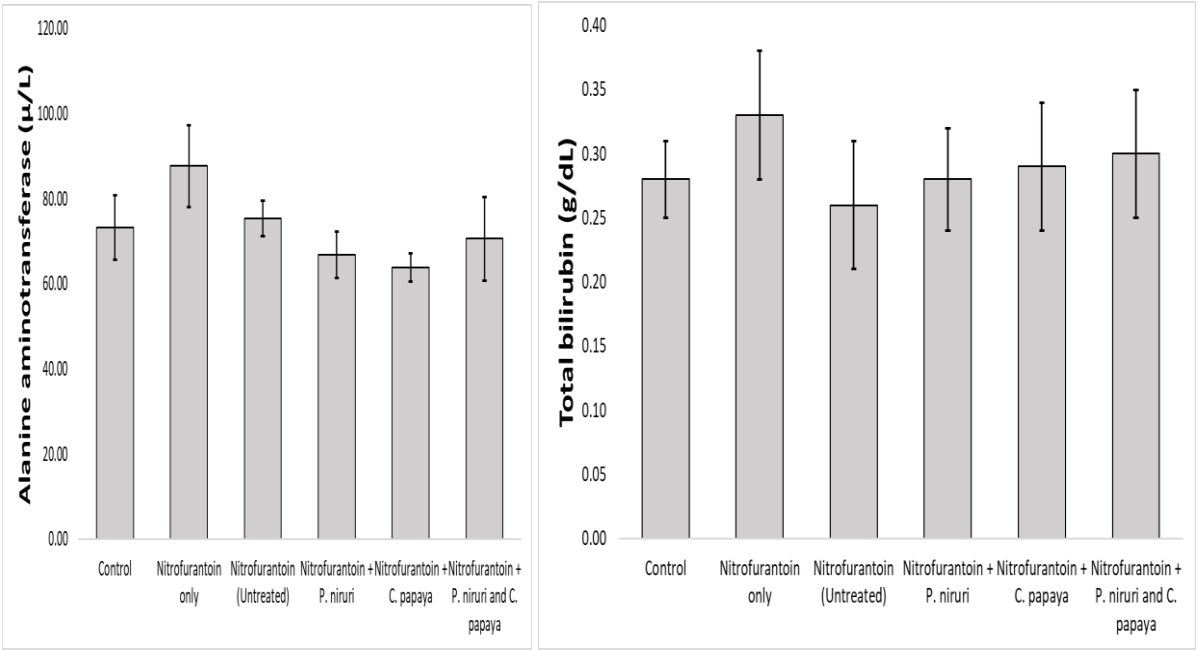

Figure 5: Chart showing levels of alanine aminotransferase and total bilirubin
Statistically significant differences (P>0.05) in alanine aminotransferase parameters across the groups, there were no statistically significant differences (P>0.05) of total bilirubin across the groups.

Weights

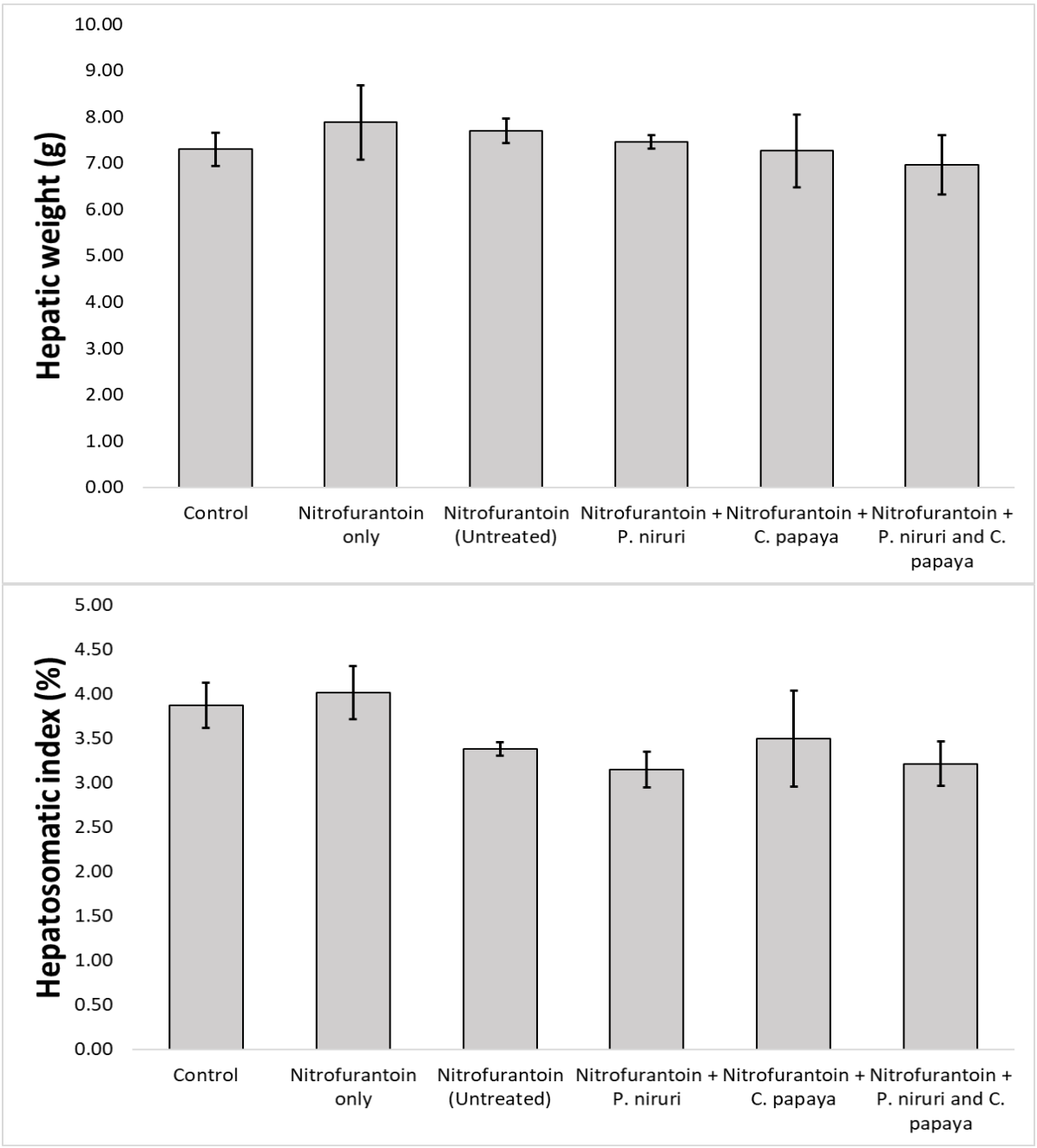

Figure 6: Chart showing hepatic weight
There were no significant differences (P>0.05) in hepatic weight across the groups.

Figure 12: Chart showing hepatosomatic index
There were no statistically significant differences (P>0.05) in hepatosomatic index across the groups.

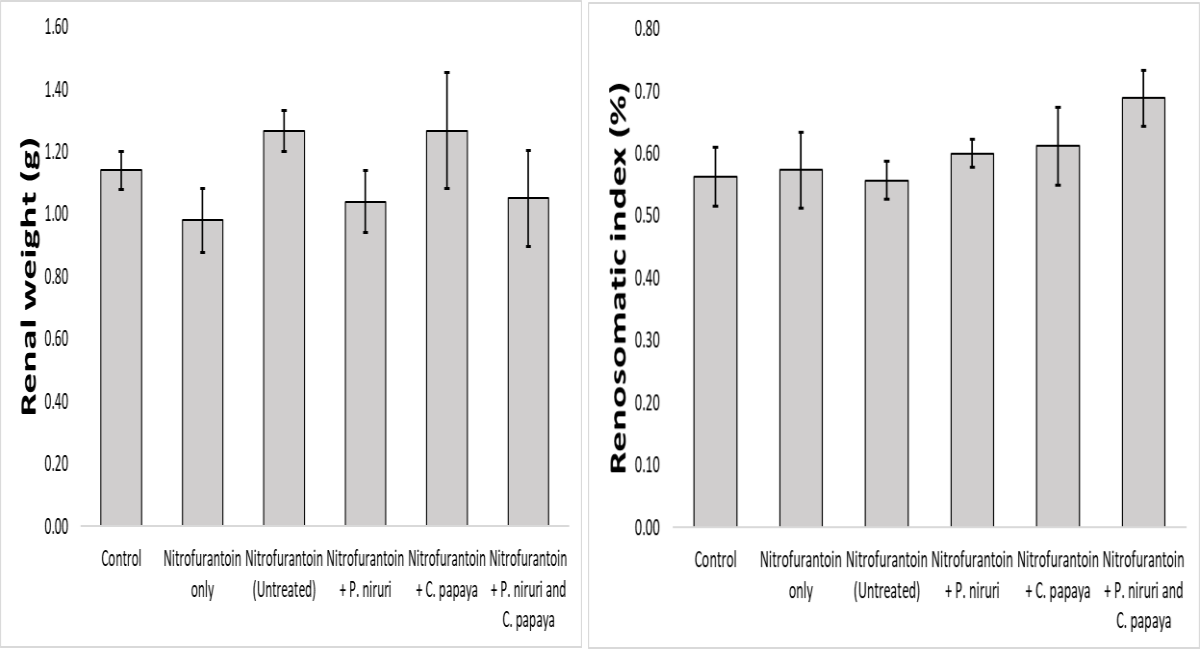

Figure 7: Chart showing renal weight and renosomatic index

There were no statistically significant differences (P>0.05) in renal weight across the groups and there were no statistically significant differences (P>0.05) in renosomatic index across the groups.

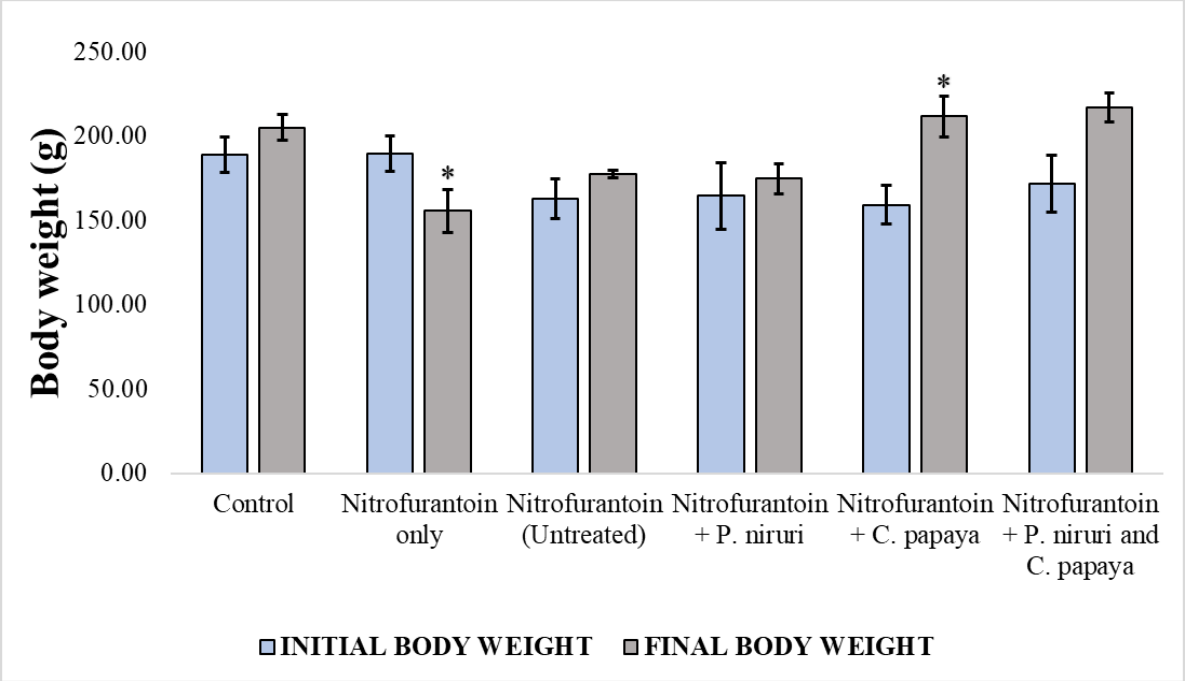

Figure 8: Chart showing body weight

*significantly different from the control group

There was a statistically significant decrease (P<0.05) of body weight in Group B, while a statistically significant increase (P<0.05) was observed in Group E, when the initial body weights were compared with the final body weights.

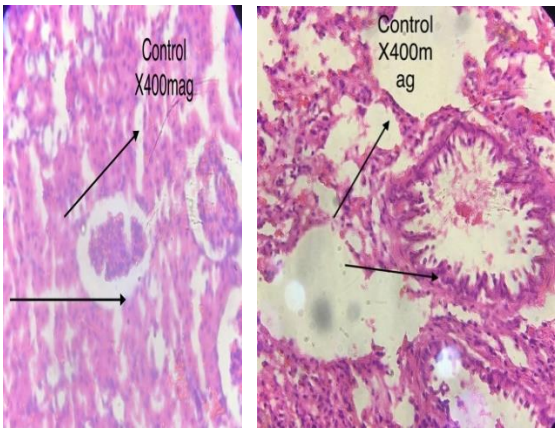

Plate 1: Photomicrograph of a control liver tissue of albino rat, fed with rat pellet and water showing normal central vein and hepatocyte (arrows), photomicrograph of a control kidney tissue of albino rat, fed with rat pellet and water showing normal bowman capsule, glomeruli, tubules (arrows revealing the cortex and medulla), Photomicrograph of a control lung tissue of albino rat, fed with rat pellet and water, showing bronchiole, bronchi and alveoli (arrow), Photomicrograph of a control lung tissue of albino rat, fed with rat pellet and water, showing remarkable bronchiole, bronchi and alveoli (arrow). H and E. X100 and 400 Mag

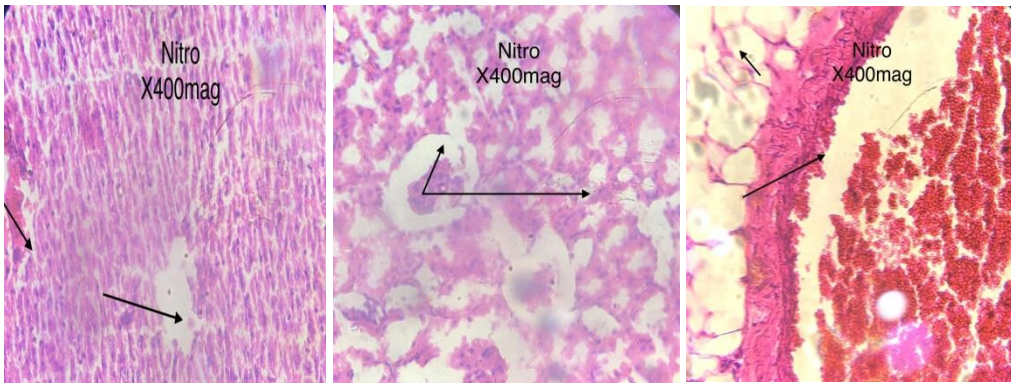

Plate 2: Photomicrograph of liver tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w of nitofuratoin showing inflammation of the hepatocyte and the capillaries slightly inflamed (arrows), photomicrograph of Kidney tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w of nitofuratoin showing depletion and slight inflammation of the glomeruli and the tubules slightly inflamed (arrows), photomicrograph of lungs tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w of nitofuratoin showing inflammation of the bronchiole and bronchi (bronchitis) and swellings (arrows). H and E. 400Mag.

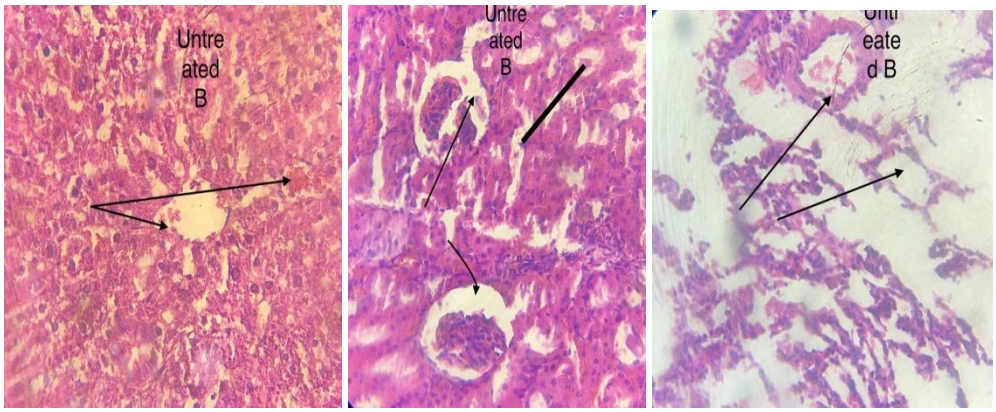

Plate 3: Photomicrograph of kidney tissue of albino rat, fed with rat pellet and water only after two weeks pretreatment with nitofuratoin showing a slightly inflamed central vein and sinusoids (arrows), Photomicrograph of kidney tissue of albino rat, fed with rat pellet and water only after two weeks pretreatment with nitofuratoin showing a slight erosion of the glomeruli (arrows), Photomicrograph of lungs tissue of albino rat, fed with rat pellet and water only after two weeks retreatment with nitofuratoin showing a slightly inflamed bronchi and steatosis of the alveoli (arrows). H and E. X400Mag.

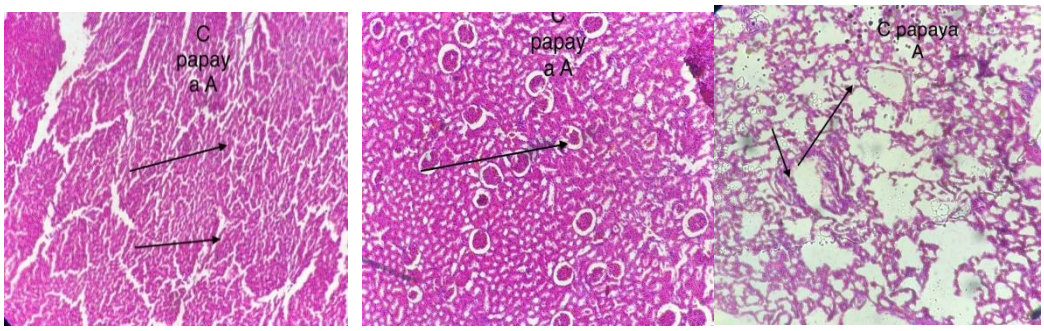

Plate 4: Photomicrograph of liver tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w and *C. papaya* after two weeks pretreatment with nitofuratoin showing a normal hepatocytes and the sinusoids (arrows), Photomicrograph of kidney tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w and *C. papaya* after nitofuratoin showing a normal glomeruli and tubules (arrows), Photomicrograph of lung tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w and C. papaya after two weeks pretreatment with nitofuratoin and showing a remarkable alveoli, bronchiole and bronchi (appearing normal) (arrows). H and E. X100 and 400Mag.

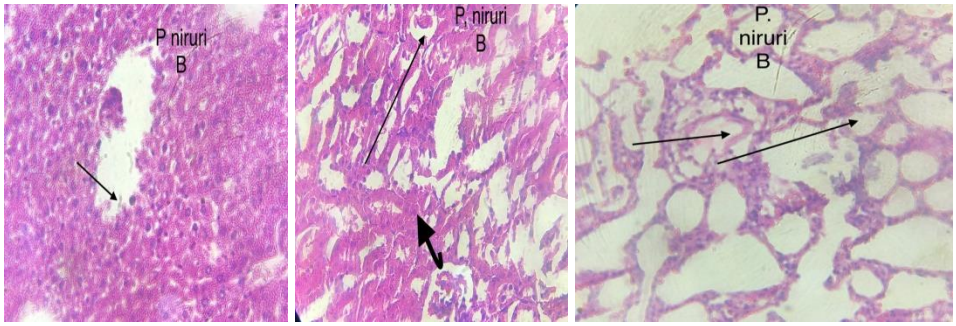

Plate 5: Photomicrograph of liver tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w, *P. niruri* after two weeks pretreatment with nitofuratoin and showing a remarkable of the hepatocytes and the sinusoids (appearing normal) (arrows), Photomicrograph of kidney tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w and *P. niruri* after two weeks pretreatment with nitofuratoin and showing a remarkable alveoli, tubules and glomeruli (appearing normal) (arrows), Photomicrograph of lung tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w, *P. niruri* after two weeks pretreatment with nitofuratoin and showing a remarkable alveoli, bronchiole and bronchi (appearing normal) (arrows). H and E. X400 Mag.

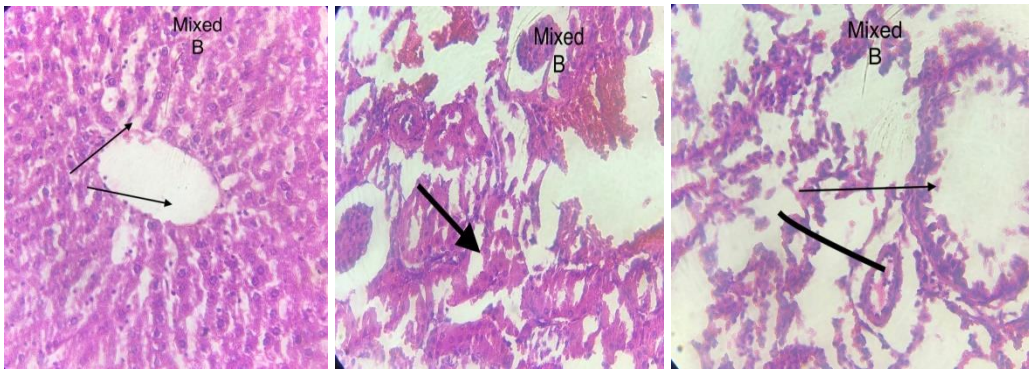

Plate 6: Photomicrograph of liver tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w, *C .papaya P. niruri* after two weeks pretreatment with nitofuratoin and showing a remarkable central vein hepatocytes and the sinusoids (appearing normal) (arrows), Photomicrograph of kidney tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w *C. papaya and P. niruri* after two weeks pretreatment with nitofuratoin, showing a normal alveoli, tubules and glomeruli (arrows), Photomicrograph of lung tissue of albino rat, fed with rat pellet and water and 100mg / kg b/w, *C. papaya, P. niruri* after two weeks pretreatment with nitofuratoin showing a remarkable alveoli, bronchiole and bronchi (appearing normal) (arrows). H and E. 400 Mag.

DISCUSSION

The values of creatinine in the groups treated with *P. niruri, C. papaya*, and both did not differ significantly (P > 0.05). Electrolytes assist in controlling kidney function.
Autumn et al., (2018), in their findings which was in line with our findings, found out that, an increase in the levels of potassium, chloride, and bicarbonate in the body results in hyperkalemia? The most common causes of hyperkalemia include chronic kidney disease, uncontrolled diabetes, and dehydration, severe bleeding from an injury, eating too much potassium, and some medications. Renal tubular acidosis may result from an increase in chloride. A high blood bicarbonate concentration can cause metabolic alkalosis, a state in which the pH of the tissues rises. Metabolic alkalosis can be brought on by the loss of acid from your body, such as through vomiting or dehydration.

Phytonutrient present in *Carica papaya* may be used in the creation of pharmaceuticals and other healthcare products (Sharma *et al*., 2020). The results of this study showed a non-significant increase in AST and ALT levels in response to the aqueous extract of *C. papaya* leaves. However, a myocardial infarction, liver disease, toxic jaundice, and other conditions could all be associated with an increase in AST and ALT levels above the normal ranges (that of the control) (Singh, 2004). This is so that significant amounts of these enzymes can leak into the bloodstream as a result of pathology affecting tissues like the liver, heart, or skeletal muscles (Pike *et al*., 2013). The amounts of these liver function enzymes in the blood plasma will increase as a result. The liver's enclosed compartments and other cells contain ALT, which is particularly helpful for spotting hepatic necrosis, especially in small animals (Ribeiro *et al*., 2019; Vagvala *et al*., 2018; Wilkerson and Ogunbodede, 2019) Since ALT is one of the specific assayable liver marker enzymes, its non-elevated level in this study suggested that administration of the crude plant leaf extract under study did not result in hepatic damage (Giannini, et al., 2005). They also discovered that after nitrofuratoin administration, aspartate aminotransferase (1444 units/L), alanine aminotransferase (1926 units/L), direct bilirubin (276 units/L).

The weight status of the test rats can also be used to infer the beneficial effects of the aqueous extract of C. papaya leaves. In comparison to the control, there were no appreciable differences in the hepatic weight, hepatosomatic index, renal weight, or reno somatic index between any of the groups (P > 0.05). (fig. 11, 12, 13, 14).

The body weight of the animals in the nitofuratoin-treated group decreased significantly (P 0.05), whereas no significant increases (P > 0.05) were observed in the untreated, C. papaya, P. niruri, and combined extract-treated groups. The significant reduction in body size may have been caused by the constituent, which could reverse after use is discontinued, as was the case with untreated rats. The fact that the test rats' weights increased non-significantly (p > 0.05) after being given Carica papaya extract suggests that the animals were in better physical condition. For instance, similar observations have been made by Nwinuka *et al*, (2008). The results of Albert and Zimmet were similar to these results (1998) According to the study's findings, the test rats' body weights were unaffected by the extracts of *C. papaya leaves, P. niruri*, and the extract because none of them caused acute fluid loss, proteolysis, or lipolysis (Albert and Zimmet, 1998; Nwiloh *et al*., 2009).

Pawpaw fruits have a juicy flavor and are rich in minerals like potassium and magnesium as well as antioxidant nutrients like carotene, vitamin C, vitamin B, flavonoids, foliate, and panthotenic acids. They are also rich in fiber, which has been linked to colon cancer prevention, cardiovascular system health, and lung rejuvenation (Fischer, 1998). The

improvement and reparative effect of the extracts from *Phyllanthus niruri, C. papaya*, and the combination of the two may be attributed to the phytochemical content of the extract, such as flavonoids and panthotemic acid.

Hepatocytes, capillaries, glomeruli, bowman capsules, renal tubules, as well as swellings (edema) around the alveoli and inflamed cells surrounding the bronchi and bronchioles (bronchitis) were all seen histologically in the liver architectures of rats given nitrofuratoin (see plates 7, 8, and 9 above). There were slight inflammations around the sinusoids and hepatocytes of the liver, glomeruli erosion in the bowman capsules, and fatty deposits around the lungs in the untreated animals (animals pretreated with nitrofuratoin without herbal treatment).

The liver, kidney, and lungs tissues recovered in the *C. papaya, P. niruri,* and combined extract treatment groups. The harmful and destructive effects inflicted on the various tissues could be reversed by the extracts. This study supports Sullivan's findings that nitrofurantoin-induced pulmonary toxicity is an acute, autoimmune condition, but there have also been reports of cases of chronic pulmonary fibrosis. In a similar vein, although nitrofurantoin-induced hepatotoxicity typically manifests as an acute condition, patients may also have active, chronic hepatitis (Sullivan, 1975), see also another study by the same author, the incidence of nitrofurantoin-induced pulmonary toxicity ranges from 0.00002 percent to 0.0009 percent, and that of nitrofurantoin-induced hepatotoxicity ranges from 0.0003 percent to 0.035 percent, according to this study. Although the combined rate of pulmonary and hepatic toxicity is unknown, it is probably lower than the combined rate of each type of toxicity separately. According to Reinhart and others, the incidence of combined toxicity is 3.9-10 per course of therapy, which could account for the dearth of cases reported in the literature (Sullivan, 1975). Similar research was done by Stricker *et al.,* (1988), who summarized the findings and noted that chronic hepatotoxicity was most frequently linked to using nitrofuratoin and also that clinical manifestations included jaundice, hepatomegaly, malaise, anorexia, losing weight, nausea, and vomiting. In roughly 70% of patients, anti-smooth muscle antibodies were discovered, and in roughly 80% of patients, anti-nuclear factors. Hepatocellular abnormalities with mixed cholestatic-hepatocellular changes were frequently found in liver biochemistry tests, and a chronic active hepatitis pattern was frequently found in liver biopsies.

CONCLUSION

Conclusively, our findings showed that nitrofuratoin can have mild to serious deleterious effect on the organs such as the kidney, liver and the lungs. The two herbal herbs have proven to be very effective, affordable and easily accessible in remedying the deleterious, hence can be used as treatment or supplements.

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