ejpmr, 2016,3(6), 61-69

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

HEPATO-RENAL TOXICITY CARICA PAPAYA EXTRACTS ON STREPTOZOCIN INDUCED DIABETES IN RATS.

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Article Received on 28/03/2016 **Article Revised on 19/04/2016** **Article Accepted on 10/05/2016**

ABSTRACT

Background: In developing countries, the traditional use of Carica papaya leaf is being investigated as an alternative to standard treatments for a range of ailments in which diabetes mellitus is one of them. Many people are aware of the protective benefits of the Carica papaya plant but there is need for its safety profile to be scientifically verified. Especially the use of the leaves which has more claimed therapeutic benefits than just for nutritional needs. Therefore, this study was aimed at assessing the nephrotoxicity and hepatotoxicity of Carica papaya leaf in streptozocin induced diabetic rats. **Methods**: Methanol, ethanol, crude and aqueous extracts of Carica papaya leaves were extracted. The phytochemical analysis of all the extracts were carried out. Thirty (30) albino rats grouped into six (6) were induced diabetes by intraperitoneal administration of 45 mg/kg of streptozotocin (STZ). Four (4) groups were fed methanolic, ethanolic, crude and aqueous extract of Carica papaya while two groups which were fed glibenclamide and normal saline as controls for fourteen (14) days. Blood sample from all the rats in each group were for biochemical analysis were collected on 14th day of administration of the extracts while kidney and liver specimens were collected for histological analysis. **Results**: The phytochemical analysis showed that all the extracts contained alkaloids, tannins, phlobatamins, flavonoids, anthraquinnones, saponins, carbohydrates and cardiac glycosides. The result showing the effect of rats fed the various extracts of Carica papaya leave on the urea and creatinine concentration were statistically non-significant ($p > 0.05$) compared to rats fed normal saline for methanolic, aqueous and crude extracts whereas those fed ethanolic extracts where significantly reduced ($p<0.05$) for urea concentration compared to control normal saline. The results showing the effect of Carica papaya extract on the hepatic indices of the rats were statistically not significantly different ($p >0.05$) compared to controls, for serum alkaline phosphatase, serum glutamate oxaloacetate transaminase and serum pyruvate oxaloacetate transaminase activities. **Conclusions**: The findings from this study shows that methanolic, ethanolic, aqueous and crude extracts of Carica papaya at the dose used in this study does not cause hepato-renal toxicity, instead it improves the recovery from inflammatory process of liver and kidney cells.

KEYWORD: Carica papaya, ethanolic, methanolic, anthraquinnones, saponins.

1.0 INTRODUCTION

Almost all the parts of Carica papaya plant is useful. Some examples include the use of the fruits, leaves and latex in brewing, wine making, textile and taming industries. A lot of popular fruit juice and beverages are also made using the ripe edible fruit. While its leaves, shoots and unripe fruits is cooked as a vegetable in some cuisine. The fruits are also used as a source of flavoring in candies, jellies, preservatives and ice creams.[1] Carica papaya has been widely used in traditional medicine for treating many ailments. Some of the tradomedical uses include the use of the juice for curing warts, cancers, tumors and thickened skin. The roots or their extracts are also, used for treatment of cancers of the uterus, syphilis, skin infection, hemorrhoids and to remove mineral concentrations in the urine.[2] Studies have confirmed that consuming papaya leaf extract can enhanced the process of wound healing due to its anti-bacterial and antioxidant actions.[3] In a related research carried out by[4] reported that aqueous extracts of Carica papaya leaf showed significantly decrease in blood glucose, cholesterol, triglyceride and amino transferase levels in diabetic rats. The skin, pulp, seeds and leaf contain a variety of phytochemicals including carotenoids and polyphenols.[5]

In developing countries, the traditional use of papaya leaf is being investigated as an alternative to standard treatments for a range of ailments in which diabetes mellitus is one of them. Studies done by scientists found that the phytochemical components in Carica papaya leaves act in synergy to display a strong antioxidant and immune enhancing impact in the blood stream. Several animals' studies and some human studies have shown

that the Carica papaya leaf and its extracts were able to demonstrate glucose lowering effect. It was discovered that Carica papaya leaf extract enhances insulin sensitivity. Poor insulin sensitivity is the cause of type 2 diabetes, which causes inefficient glucose uptake by the cells.[6]

Many people are aware of the nourishing restorative and protective benefits of the Carica papaya plant but there is need for its safety profile as a therapeutic agent to be scientifically verified. Especially the use of the leaves which has more claimed therapeutic benefits than just for nutritional needs. The objective of this study is to assess the nephrotoxicity and hepatotoxicity of Carica papaya leaf.

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Materials

The leaves of Carica papaya was purchased from mile three (3) market in Port Harcourt, Rivers State and was authenticated by a botanist in the department of biology in Rivers State University of Science and Technology, Port Harcourt, Rivers State Nigeria.

2.2 Preparation of Plant Extract

The plants leaves were allowed to air dry for one (1) week. The dried leaves were ground into powder with an electric blender. The various extracts from methanol, ethanol and water was prepared using the hot continuous extraction method known as soxhlet extraction. While the crude extract was gotten using cold maceration method.

2.2.1. Hot Continuous Extraction (Soxhlet)

50g of the finely ground plant leaves was placed in a thimble, which was placed in the chamber of the soxhlet apparatus. While, 100ml of the extracting solvent was placed into the flask. The solvent was then heated to different temperatures depending on the boiling point of the extracting solvent, 100oc for aqueous, 78.3^{o}c for ethanol and 64.7^{o}c for methanol. The vapor from the solvent then moved up to the column and floods into the chamber containing the thimble with the dried plant leaves. The non-volatile compounds present in the leaves was then dissolved into the solvent which condensed back into the flask. This process was repeated until all the desired concentrated compounds in the plant in the chamber had been extracted. The extract and the solvent mixture was then put into a Rotary evaporator to be able to separate the extract from the solvent by evaporating the solvent from the mixture. After all the solvents had been removed the extract was finally placed on a water bath to remove all the moisture content. This was done at 45^{o}c so as not to denature the plant constituents.

2.2.2. Cold maceration

In preparing the crude plant cold maceration was used. 570g of the finely ground dried plant leaves was immersed into the 1000ml of distilled water for 72 hour. It was then filtered using a Whatman filter paper. The water content of the extract was then evaporated by drying the mixture on a water bath at 45^{o}c.

2.3 Phytochemical screening

The various extracts were screened for phytochemical constituents using the following procedures:

2.3.1. The procedure used to screen for alkaloids Test

To screen for the presence of alkaloids in the various extracts, 5grams of each extract was put into a test tube containing 5% hydrochloric acid and heated for 5min. After cooling they were
filtered into three different test tubes for each extract. Mayers, Hagers and Drangendorffs reagent
were then added into each test tube. A yellow and creamy precipitation was observed for those
that contained alkaloids.

2.3.2. The procedure used to screen for tannins Test

To screen for the presence of tannins in the various extracts, 1 ml of each extract was treated with 5% of ferric chloride reagents. A blue-black precipitate was observed, in those that contained tannin.

2.3.3. The procedure used to screen for phlobatannins Test

To screen for phlobatannins two methods were used the Hydrochloric acid test and Formaldehyde test methods. In the hydrochloric test 0.5 grams of extract was dissolved in water then filtered, the filtrate was boiled with 1% hydrochloric acid for 5min, and those that contained phlobatannins produced red coloured precipitate. While, in the second method 40% of formaldehyde and 6 drops of 1% hydrochloric acid were added to the filtrate and heated. Then allowed to cool. Then 5% potassium hydroxide was added to the cooled solution. Those that produced a bulky precipitate contained phlobatannins.

2.3.4. The procedure used to screen for anthraquinnones Test

To screen for Antraquinnones, the Bontragers test for free antraquinnones method was used. The extract were shaken with chloroform, then filtered. The filtrate was treated with 10% ammonia solution. Those that when shaken showed a pink colouration contained free anthraquinnones. While, to screen for Anthraquinnones Combine, the extracts were heated with 10ml dilute sulphuric acid and allowed to cool and filtered. The filtrate was added to 5mls of benzene and 10% ammonia solution. A violet colouration was noted in those positive result.

2.3.5. The procedure used to screen for flavonoids test

To test for flavonoids the shinoda reduction method was used. The extracts were dissolved in Hydrochloric acid and then filtered. Few pieces of magnesium metal were added to 5mls of the filtrates. Those that formed orange or red crimson colour, were noted as a positive result.

2.3.6. The procedure used to screen for Carbohydrate test

To screen for simple sugars which are component of glycosides. A dilute solution of about 2% of the sugar solution was prepared in water, and to two ml of this solution, 2 drops of 10% alcoholic solution of alpha-naphtol, was added into a test tube. The test tube was warmed in a hot plate to 45^0c. Then carefully concentrated sulphuric acid was added. Those that a deep violet ring was produced where the liquids met indicated they contained insoluble carbohydrate. The result was taken as presence of glycoside. The Fehling test was also performed still to screen for the presence of reducing sugars. With Fehling solution for sugar reduction, the extract after filtering with water was treated with Fehling solution A+B after boiling for about 5min and allowed to cool. Concentrated sodium bicarbonate was added. Those that the sodium bicarbonate did not change the colour of the solution contained reducing sugars.

2.3.7. The procedure used to screen for saponin glycosides

The Saponin test for frothing was used which was by Soforowa at 1993. This is a preliminary test for the evidence of saponin, the plant extracts were shaken with distilled water vigorously for 2min, and allowed for 10-15min for observation if frothing (foaming). Those with persistent frothing within this period were said to contain saponin

2.3.8. The procedure used to screen for Cardiac glycoside

To screen for cardiac glycoside Acetic acid was used to dissolve the plant extract together with conc. H_2S0_4 to form a layer. Those that the colour gradually changed from violet to green indicated the presence of cardiac glycosides.

2.4. Collection of Animals

The protocol employed met the guidelines of the Good Laboratory Practice (GLP) regulations of World Health Organization. Forty (45) albino rats of both sexes weighing between 190 and 240 g were purchased from an animal farm house in Enugu, Nigeria. The rats were allowed to acclimatize for 14 days in the laboratory in the animal farm house in the Department of Human Physiology, University of Port Harcourt, Rivers State. The rats were fed with normal rat feed and water ad libitum. 10 rats were used for a pilot study to ascertain the amount of streptozocin that could induce diabetes in the rat without being lethal. While the 30 rats left which were randomly grouped into 5 groups with five rats in the groups as shown in the table 2.1 below.

Table2.1: Grouping of experimental rats

s/no	Carica papaya
1	Methanol extract group
2	Ethanol extract group
3	Aqueous extract group
4	Crude extract group
5	Control 1(glibenclamide)
6	Control 2 (normal saline)

2.5 INDUCTION OF DIABETES

2.5.1. Induction of the rats

Thirty five (35) albino rats were injected a 45mg/kg bodyweight of streptozocin shown by the pilot study to induce diabetes without being lethal to them. The fasting blood glucose level of each rat was taken before injecting the rats with streptozocin to form a baseline glucose level of the rats which the mean was discovered to be 3.5mmol/l. The animals were fed freely with normal rat feed and water during the day but an overnight fast was maintained so that the fasting blood glucose level could be monitored every morning. A rise of the fasting blood glucose level was noted to occur on the 5^{th} day after induction of Diabetes. The mean value was 6.6 mmol/l. 5 rats did not become diabetic at that day but as the average number of rats were already diabetic the administration of the extracts had to commence. So such rat were removed from the study.

2.6 Administration of Plant Extracts

Treatment of diabetic rats with the crude plant and various plant extracts commenced on the 6^{th} day, after streptozocin administration. 0.7mls of the extract was be administered orally for 2 weeks. This amount was calculated by using the median dose which is 200mg/kg.

2.7 The Laboratory Analysis

The blood samples for nephrotoxicity test and hepatotoxicity test was collected on 14th day of administration of the extracts. The samples were collected from the animals sacrificed under 70% chloroform anesthesia into a plain specimen bottle. The samples were allowed to clot, then centrifuged at 3000 revolutions per minute for 3 minutes. The sera obtained were stored in a freezer until required for use in analysis. The serum collected was used for biochemical analysis of alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) using the Elitech reagent kit following the continuous monitoring assay methods of kinetics. While the urea and creatinine were determined colorimetrically, also using Elitech reagent kit following the method described by Teitz. The samples were assayed using the Selectra PROS chemistry auto-analyzer with quality controls, following the required standard operating procedures (SOP's).

2.8 Histological studies

On the 14^{th} day of administration of the extracts to the streptozocin induced diabetic rats one from each group was randomly chosen that was dissected to obtain the

kidney and liver. The samples collected were placed in 10% formal saline for histological examination at the University of Port Harcourt Teaching Hospital Histology Laboratory.

2.9 Statistical studies

The results were analysed using SPSS version 20. One-way analysis of variance (ANOVA) was used to compare means followed by Post Hoc LSD multiple comparison test where p-values were significant. P-value < 0.05 was considered statistically significant.

3.0 RESULTS

Table 3.1: Phytochemical analysis of various extracts of Carica papaya Leaves

	Methanol extract	Ethanol extract	Aqueous extract	Crude extract
Alkaloids test:				
a) Mayers reagent test	+ve	++ve	++ve	+ve
b) Hagers reagent test	+ve	++ve	++ve	+ve
c) Drangendoffs reagent test	+ve	++ve	++ve	+ve
Tannins test:				
a) Feric chloride test	+ve	+ve	+ve	+ve
b) Bromine water test	+ve	+ve	+ve	+ve
Phlobatamins test:				
a) Hydrochloric test	+ve	-ve	-ve	-ve
b) 40% formaldehyde test	+ve	-ve	-ve	-ve
Anthraquinnones:				
a) Free anthraquinnones	-ve	-ve	-ve	-ve
b) Combine anthraquinnones	-ve	-ve	-ve	-ve
Flavonoids test:				
a) Test for shinoda reduction	-ve	++ve	+ve	-ve
Carbohydrate test:				
a) Molisch test	+ve	+ve	+ve	-ve
b) Fehling test for sugar reduction	-ve	++ve	+ve	-ve
Saponin Glycosides test:				
a) Frothing test	-ve	++ve	+ve	-ve
b) Emulsion test	-ve	+ve	+ve	-ve
Cardiac glycoside test:				
a) Kedde test	-ve	++ve	+ve	-ve
b) Salwoski test	-ve	++ve	+ve	-ve
Steroidal/ steroid test:				
a) Keller killiani test	-ve	++ve	+ve	+ve
b) Lieberman test	-ve	++ve	+ve	+ve

3.2 Toxicity studies

3.2.1 Nephrotoxic studies

Table 3.2: Evaluation of day 14 Urea and Creatinine levels in diabetes rats treated with various solvent media of Carica papaya leave extracts

GROUPS	UREA (mg/dl mean ± SEM)	CREATININE Mg/dl mean ± SEM)
methanol	49.0 ± 2.39	0.46 ± 0.03
Ethanol	32.60 ± 1.50*	0.57 ± 0.03
Aqueous	33.60 ± 2.09	0.44 ± 0.01
Crude	36.40 ± 2.93	0.44 ± 0.02
Control 1(gliben)	38.80 ± 0.49	0.52 ± 0.05
Control 2(saline)	42.80 ± 3.15	0.38 ± 0.01

Values are presented as mean ± SEM. n= 5, * mean values are statistically significant compared to the control (saline)

Table 3.2 is showing the results of the urea and creatinine concentrations of streptozocin induced diabetic rats fed with methanol, ethanol, aqueous and crude extract of Carica papaya leaves after fourteen (14) days. The result showing the effect of rats fed the various extracts of Carica papaya leave on the urea and creatinine concentration were statistically non-significant (P> 0.05) compared to rats fed normal saline for methanolic, aqueous and crude extracts whereas those fed ethanolic extracts where significantly reduced (P<0.05) for urea concentration and significantly (P< 0.05) increased for creatinine compared to control normal saline.

3.2.2 Hepatotoxic studies

Table 3.3: Evaluation of some liver enzyme level in diabetes rats treated with various solvent media of Carica papaya extracts

GROUPS	ALP (U/L ± SEM)	GOT (U/L ± SEM)	GPT (U/L ± SEM)
methanol	549.40 ± 32.80	127.0 ± 66.94	55.40 ± 33.31
Ethanol	524.60 ± 36.53	315.6 ± 20.44	83.0 ± 33.68
Aqueous	590.60 ±133.91	319.60 ± 85.29	88.80 ± 20.50
Crude	391.80 ± 53.65	217.20 ± 94.24	98.75 ± 23.32
Control 1(gliben)	726.20 ±115.53	336.60 ± 63.61	73.80 ± 23.14
Control 2(saline)	654.40 ± 58.54	37.0 ± 14.70	2.80 ± 0.49

Values are presented as mean ± SEM. n= 5, * mean values are statistically significant compared to the control (saline). Table 3.3 is showing the results of the serum alkaline phosphatase, serum glutamate oxaloacetate and serum pyruvate oxaloacetate concentrations of streptozocin induced diabetic rats fed with methanol, ethanol, aqueous and crude extract of Carica papaya leaves after fourteen (14) days. The results showing the effect of Carica papaya extract on the hepatic indices of the rats were statistically not significantly different (P >0.05) compared to controls, for serum alkaline phosphatase, serum glutamate oxaloacetate transaminase and serum pyruvate oxaloacetate transaminase activities.

3.2.3 Histological studies

Pictures showing the histological findings of the liver and kidney harvested from one rat from each of the various groups are shown below.

Histological studies in the liver of various groups

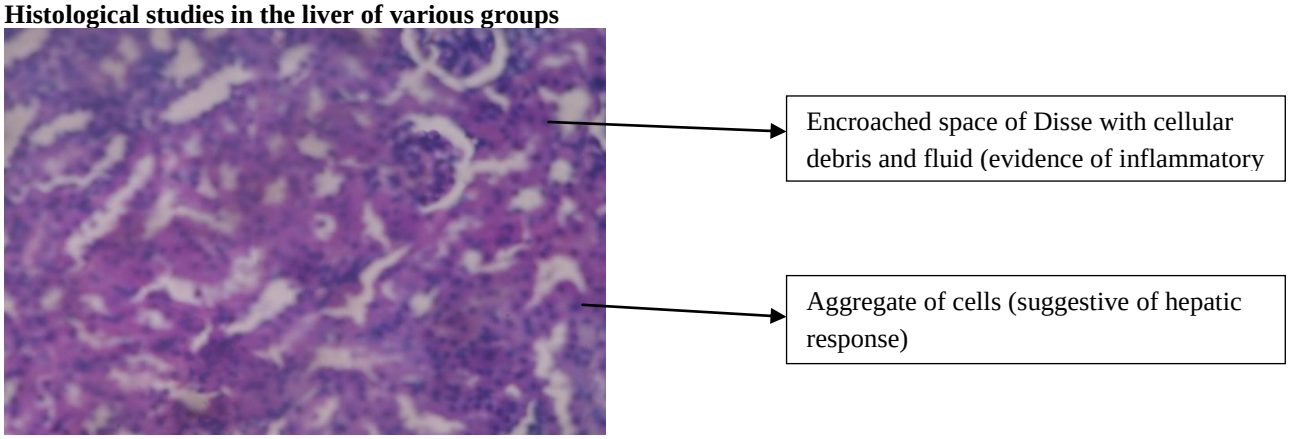

Fig 4.4.3.1a: Photo micrographic slide of liver organ of group (control saline) H & E X400

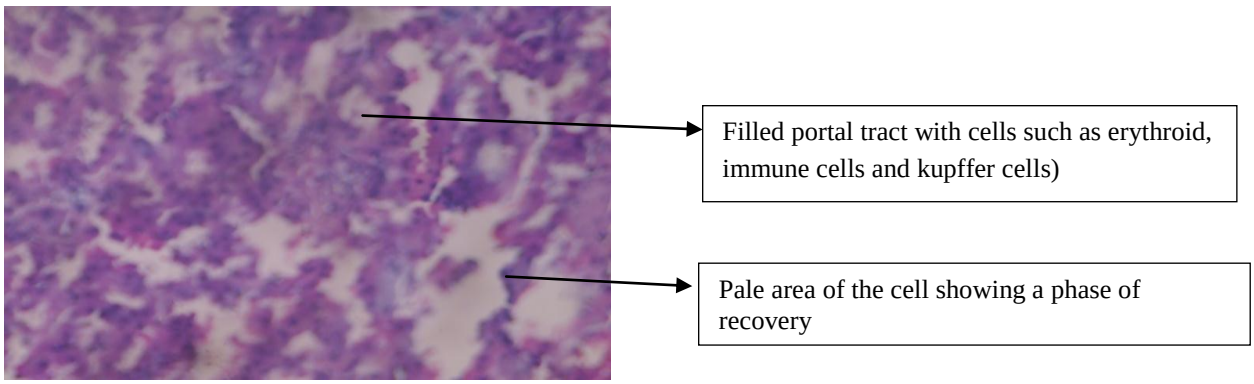

Fig 4.4.3.1b: Photo micrographic slide of liver organ of group 2 (positive control drug) H & E X400

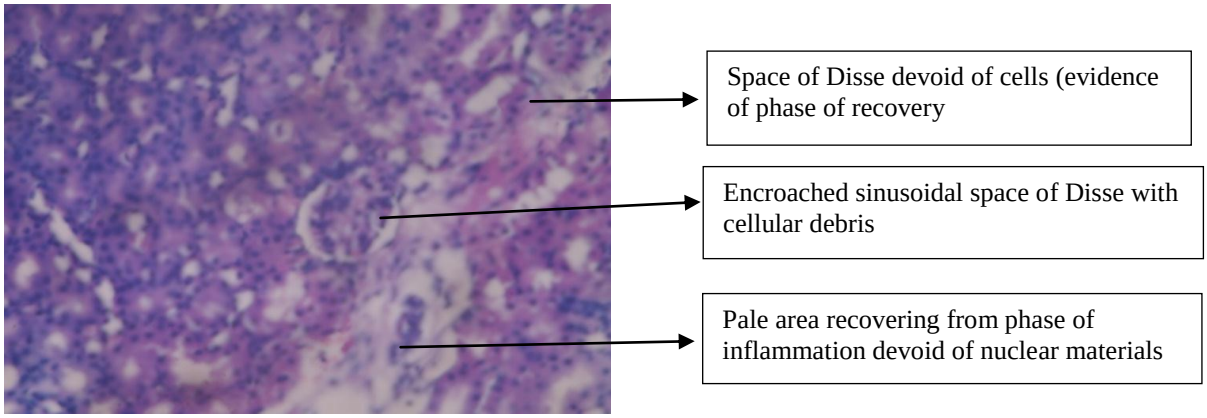

Fig 4.4.3.1d: Photo micrographic slide of liver organ of group 3 using methanolic extract of Carica papaya (200mg/kg). H & E X400

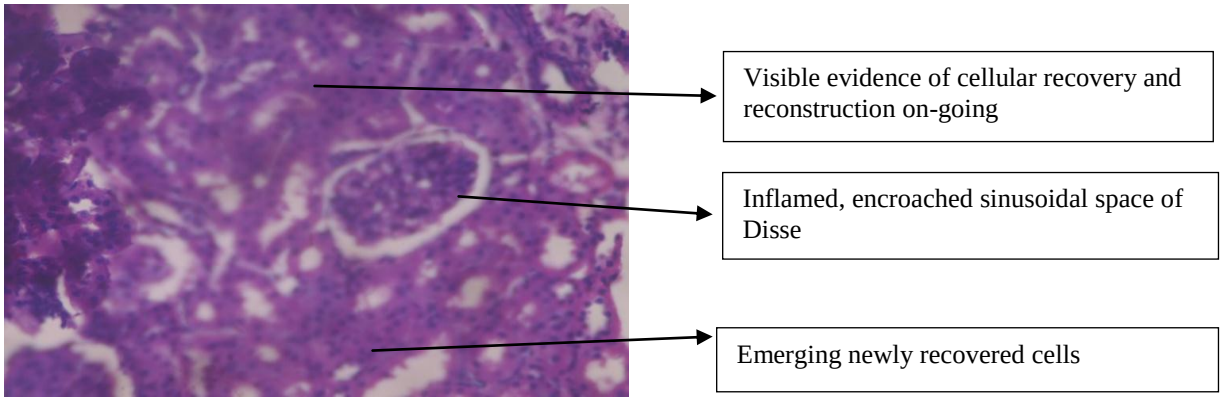

Fig 4.4.3.1f: Photo micrographic slide of liver organ of group 4 using ethanolic extract of Carica papaya (200mg/kg). H & E X400.

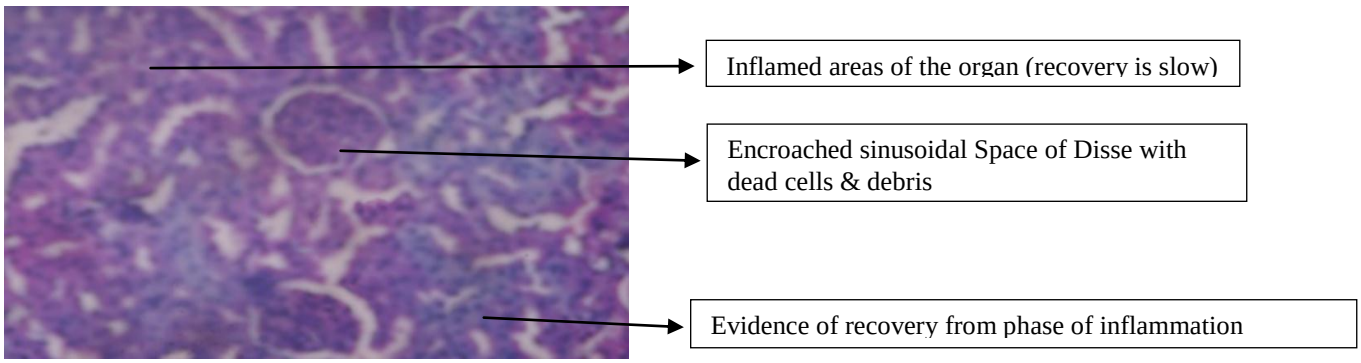

Fig 4.4.3.1h: Photo micrographic slide of liver organ of group 6 using aqueous extract of Carica papaya (200mg/kg). H & E X400.

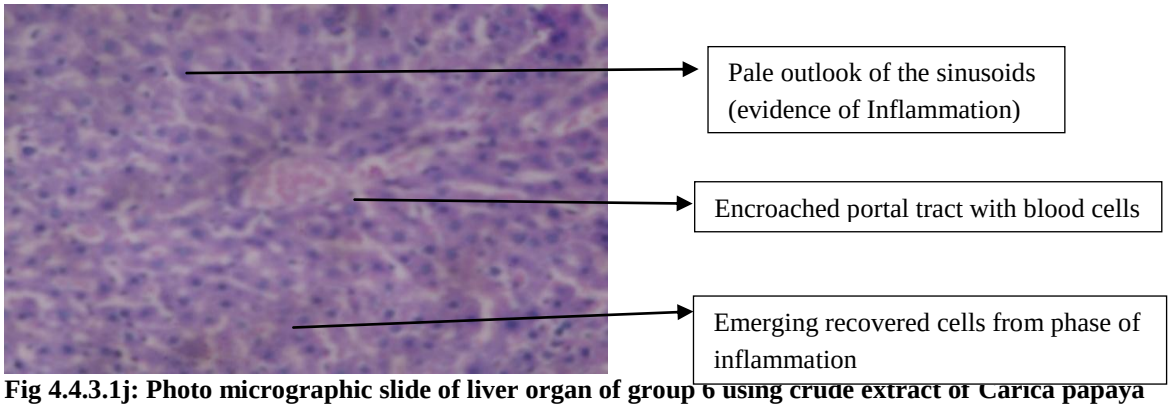

Fig 4.4.3.1j: Photo micrographic slide of liver organ of group 6 using crude extract of Carica papaya (200mg/kg). H & E X400.

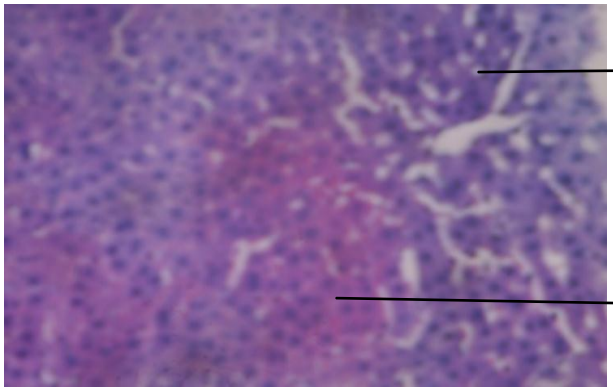

Fig.4.4.3.2a: Photo micrographic slide of kidney organ of group (control saline)

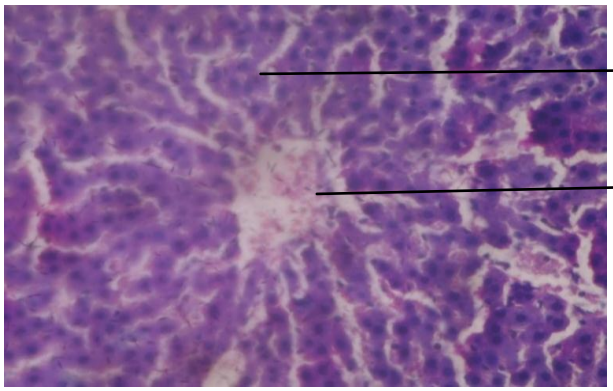

Fig 4.4.3.2b: Photo micrographic slide of kidney organ of group 2 (positive control drug)

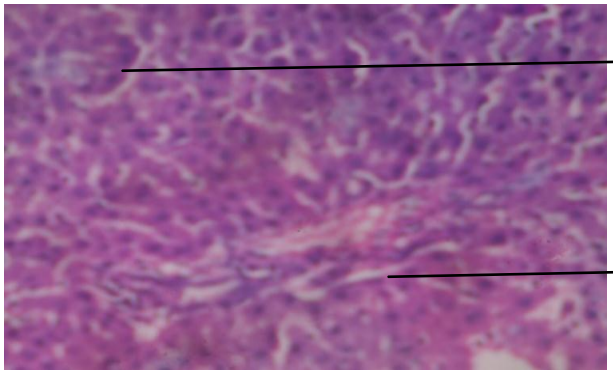

Fig 4.4.3.2d: Photo micrographic slide of kidney organ of group 3 using methanolic extract of Carica papaya (200mg/kg)

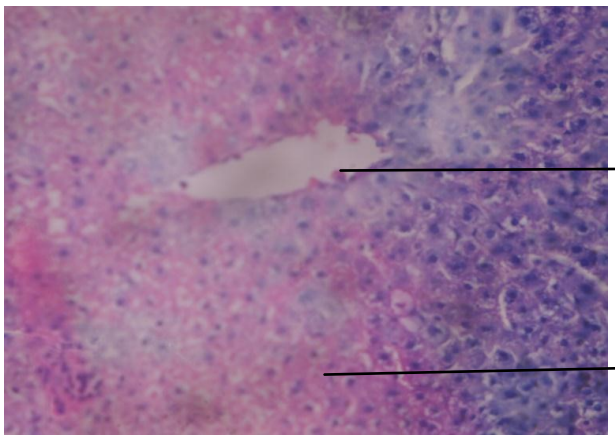

Fig 4.4.3.2f: Photo micrographic slide of kidney organ of group 4 using ethanolic extract of Carica papaya (200mg/kg)

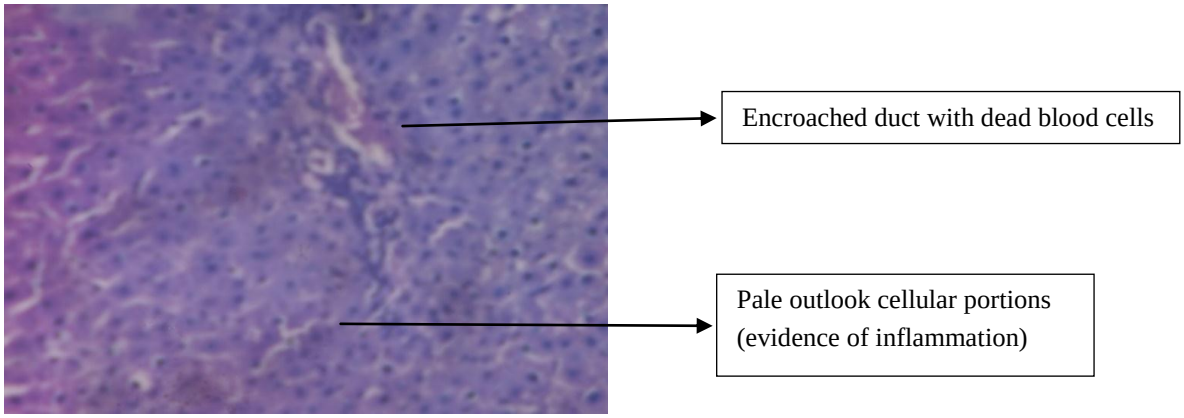

Fig 4.4.3.2h: Photo micrographic slide of kidney organ of group 5 using aqueous extract of Carica papaya (200mg/kg)

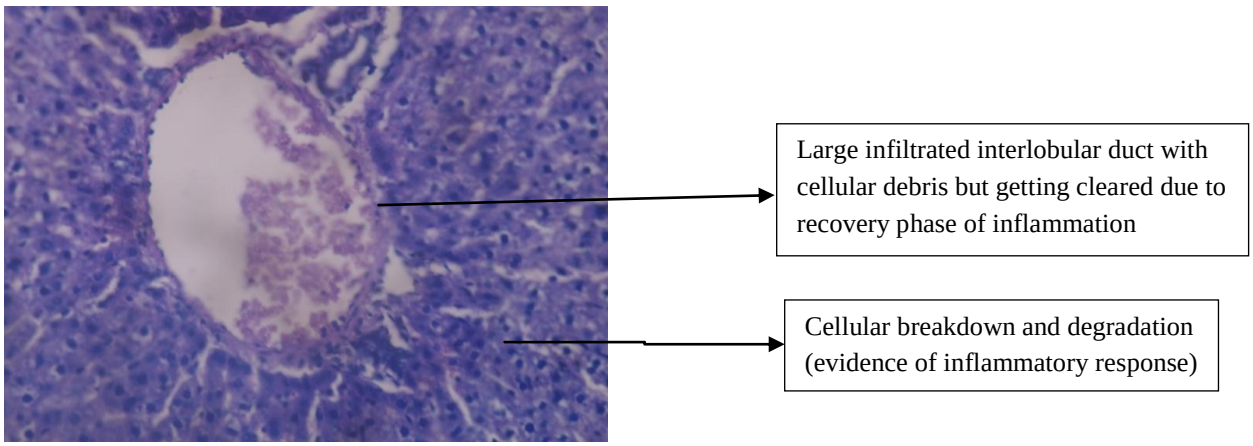

Fig 4.4.3.2j: Photo micrographic slide of kidney organ of group 6 using crude extract of Carica papaya (200mg/kg)

4.0 DISCUSSION

Africa is blessed with a lot of fruits and vegetables which are being harnesses for their therapeutic purposes. These fruits and vegetables are known to contain bioactive constituents known as phytochemicals. The phytochemicals are associated with the therapeutic benefits of the plant. From the phytochemical analysis done in this study it was observed that Carica papaya contains some of these phytochemicals such as phenols, flavonoids, tannins, alkaloids, saponins, cardiac glycosides and anthraquinnones which from previous studied have been reported of showing hypoglycemic and anti-inflammatory properties.[7] Blood urea and creatinine are biomarkers of kidney dysfunction.[8] However, the administration of all the various extracts of Carica papaya did not increase the urea and creatinine concentration of the rats. Alkaline phosphatase, serum glutamate oxaloacetate transaminase, serum pyruvate oxaloacetate transaminase are considered as one of the liver toxicity markers.[9] The various extracts of Carica papaya leaves did not cause any significant increase in these hepatic indices of the rats instead there was a significant decrease in the serum pyruvate oxaloacetate transaminase activities. This is in agreement with the study on Mauritian population which showed that Carica papaya leaf extract supplementation decreased the enzyme levels of ALT and AST (Alanine amino transferase and aspartate amino transferase) which are type 2 diabetes biomarker among diabetic patients and improved insulin sensitivity.[2] Carica papaya aided in the kidney and liver cells healing manifested by the recovery from inflammatory response phase shown in the phot micrograph slides. There are other studies that confirmed consuming papaya leaf extract can enhanced the process of wound healing due to its anti-inflammatory properties.[2,10]

5.0 CONCLUSION

The findings from this study shows that methanolic, ethanolic, aqueous and crude extracts of Carica papaya at the dose used in this study does not cause hepato-renal toxicity, instead it aids in recovery from toxic damage of the cells of the liver and kidney.

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