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Antibacterial Activity and Toxicological Properties of Ethanolic Leaf Extract from *Carica papaya*

Ayomide Olubunmi Omojokun*, Muftau Kolawole Oladunmoye

Department of Microbiology, Federal University of Technology, Akure, Nigeria

Email address

aoomojokun@gmail.com (A. O. Omojokun)

*Corresponding author

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Abstract

This study was carried out to investigate the antibacterial activities and toxicological properties of the ethanolic leaf extract from pawpaw (*Carica papaya*). The phytochemical screening of the extract showed that cardenolides and saponins are present in the leaf extract. The extract was tested for antibacterial activities against some clinical microorganisms such as *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pyrogenes, Escherichia coli, Pseudomonas aeroginosa*and *Enterobacter aerogenes*. The test was carried out using the agar well diffusion method. The *in- vivo* assay of the experiment was carried out on albino rats. This was done in order to determine the toxicological effect of the plant extract on mammals. The result showed that the plant extract has antibacterial effect against some of the organisms at different concentrations, the zones of inhibition also increased with increased concentration. It was observed that *Klebsiella pneumonia, Streptococcus pyrogenes* and *Enterobacter aerogenes* were not inhibited in all the concentrations of the extract. At 150mg/ml of the extract, *Pseudomonas aeroginosa, Escherichia coli* and *Staphylococcus aureus* had zones of inhibition of 8.0, 8.0 and 6.0mm respectively. The weight of the rats decreased during infection while there was significant increase in their weight when the extract was administered to them. The haematological studies carried out on their blood sample revealed that there was decrease in the percentage of erythrocyte sedimentation rate, packed cell volume, haemoglobin estimation, red blood cell count and lymphocyte during infection while there was increase in the percentage of white blood cell, neutrophils, eosinophils, monocytes and basophil during infection. The result obtained for the treated is close to that of the control. The histopathological analysis showed that the heart of rat infected with *S. aureus* had necrosis, slight hemorrhage and deep vacoulation of heart striata. Photomicrograph of heart of rat infected with *P. aeruginosa* revealed divergence of heart striata with necrotic edge while the photomicrograph of the intestine of rat infected with *E. coli* showed washed intestinal villi and hemorrhagic edge. The treated rats had recuperating heart and intestine which is close to that of the control.

Keywords

Carica papaya, Antibacterial, Haematological, Histopathological

1. Introduction

Pawpaw (Carica papaya) is a herbaceous succulent plant which belongs to the family Caricaceae. It is believed to have originated from Mexico and South America, although pawpaw is now cultivated in most tropical countries [1]. The plant is perennial with about 2-10m height. It usually has a single, soft, hollow, erect stem, with no branch. The leaves are directly attached to the stem. The fruit shape varies from oval to round or elongated club-shaped. The fruit size varies from 15-50cm long, 10-20 cm thick and 1-3 kg in weight [2]. The mature unripe fruit has a greenish colour which changes to yellow or orange colour as the fruit ripens. The flesh of the ripe fruit is deep yellow in colour with numerous small black, peppery seeds at the central hollow of the fruit [2]. The ripe fruit is usually peeled and eaten raw. Cooked green pawpaw fruit and leaves are also consumed in some countries [3]. Pawpaw seeds are edibile, they have a sharp, spicy taste and are sometimes ground to substitute for pepper [4]. Unripe

pawpaw is also used as meat tenderizer.

Different parts of pawpaw (skin, pulp, seeds, leaf, and bark) have been reported to contain different phytochemicals [5]. *Carica papaya* is a source of calcium, vitamin A and C. Pawpaw leaves is often used for malaria treatment [6]. Different parts of the plant are employed in the treatment of various human and veterinary diseases in various parts of the world. Extracts from pawpaw fruits and seeds have been reported to possess bactericidal activity. Its seeds possess antiparasitic properties. The plant has also been reported to have sedative, muscle relaxant, reversible antifertility and purgative properties [1].

Phytochemicals from *Carica papaya* are increasingly investigated and have shown great pharmacological potential as a result of their antioxidant, antiinflammaatory and antimicrobial properties [7]. This research therefore is aimed to determine the antibacterial and toxicological properties of ethanolic leaf extract from *Carica papaya.*

2. Methodology

2.1. Collection of Samples

Carica papaya leaves were obtained from Akure, Nigeria. The test organisms used were all human pathogenic organisms of clinical origin. These isolates include *Escherichia coli, Pseudomonas aeruginosa, Klebseilla pneumoniae, Staphylococcus aureus, Enterobacter aerogenes* and *Streptococcus pyrogenes*. They were obtained from the Department of Microbiology and Parasitology laboratory, State Specialist Hospital, Akure, Ondo- state, Nigeria.

2.2. Standardization of Test Organisms

Mcferland standard was used to standardize the isolates as decribed by Oyeleke *et al.* [8].

2.3. Processing of Plant

Carica papaya leaves were washed with clean water and air-dried for four (4) weeks. The dried leaves were ground using a marlex blender and stored in an air tight container.

2.4. Extraction of Plant

The ground leaves was soaked in ethanol after which the bioactive ingredients was extracted using a rotary evaporator. The extract was reconstituted with 30% Dimethyl Sulphuroxide ($DMSO_4$) and filtered in a whatmann filter paper. This was later purified using a 4µm membrane filter paper [9]

2.5. Phytochemical Screening

The extract was screened for the presence of bioactive ingredients such as alkaloids, tannins, saponin, antraquinone and cardiac glycoside according to the method described by Adesanya and Sofowora [10].

2.6. Preparation of Cells of the ISOLATES

The organisms were transferred from an agar slant into 10ml Nutrient broth using a sterile inoculating loop, mixed well and incubated at 37°C for 24 hours. After incubation, the content was centrifuged at a speed of 3000 revolution per minute (rpm) for 10 minutes to harvest the cells. The supernatant was discarded and the harvested cells were washed with sterile distilled water and re-centrifuged, the cells were then re-suspended in 10ml sterile distilled water which served as the stock solution for serial dilution.

2.7. Measurement of Weight of the Albino Rats

The weight of the albino rats was measured by the use of a sensitive weighing balance (ATOM-A110C (platinum)). The weight was taken every two days and the weights were recorded in grams.

2.8. Determination of the Infectivity Dose

This was carried out according to the method of Willey *et al*. [11] with slight modifications. Sterile test tubes were arranged serially, 9ml sterile distilled water was dispensed into 6 set of test tubes, and 1ml of the stock (cell) solution was introduced into the first tube making 1:10 dilution. This procedure was repeated for the remaining 5 tubes. One ml of each dilution was pour plated on nutrient agar medium. The plates were incubated at 37°C for 18-24 hours. Visible colonies were counted and estimated according to the dilution factor. The rats were challenged orogastrically with 1ml of the different corresponding dilutions. They were observed for 1 week for any clinical symptom of infection. The dose that was able to produce the highest clinical effect on the animal was calculated and used as the infectivity dose of the organism.

2.9. Animal Bioassay

Organisms that tested positive in the *in vitro* assay were used. The rats were orogastrically dosed with the infectivity dose of the different organisms. The rats were treated with the plant extract orally for 14 days (2 weeks) while uninfected rats served as the control. The blood of the rats were collected using cardiopuncture and the heart and intestine were dissected out before, during infection and after treatment with the leaf extract.

2.10. Haematology and Histopathology Assay

Blood samples from the rats were analyzed for packed cell volume (PCV), erythrocyte sedimentation rate (ESR), haemoglobin estimation (HB), white blood cell count (WBC), red blood cell count (RBC) and white blood cell differential count. The WBC differential counts include neutrophil, eosinophil, basophil, lymphocyte and monocyte. [12]. Histopathological analysis was carried out on the heart and intestine of the test animals. This was done by the method of Alturkistani, *et al.* [13].

3. Results

3.1. *In- vitro* Activity of *Carica papaya* Leaf Extract on the Organisms

The plant extract had inhibitory effect on some of the organisms. From the experiment, it was observed that not all the organisms were inhibited by pawpaw leaf extract. It was also observed that inhibitory effect was enhanced by increased concentration of extract. When 50mg/ml of the extract was tested against the organisms, only *Klebsiella pneumoniae* and *Escherichia coli* had zones of inhibition of 2.0 mm. When the concentration was increased to 100mg/ml, the extract inhibited *Klebsiella pneumoniae* by 4.0 mm, *Escherichia coli* was inhibited by 6.0 mm while *Staphylococcus aureus* was inhibited by 4.0 mm. There was no zone of inhibition in *Pseudomonas aeruginosa, Streptococcus pyogenes and Enterobacter aerogenes.* At 150mg/ml of the extract, *Klebsiella pneumonia* had inhibition of 8.0mm, *Escherichia coli* had inhibition of 8.0 mm while *Staphylococcus aureus* had inhibition of 6.00 mm. at 200mg/ml of the extract, *Klebsiella pneumonia* had inhibition of 12.0mm, *Escherichia coli* had inhibition of 10.00mm and *Staphylococcus aureus* had inhibition of 10.0mm. This result is shown in table 3.

3.2. Minimum Inhibitory Concentrations

The least concentration of the ethanolic extract of *Carica papaya* that inhibited *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* are 50mg/ml and 100mg/ml respectively.

3.3. Determination of the Infectivity Dose

Clinical parameters such as stool inconsistency, rate of feeding, agility (active/ weak) and skin were observed. It was observed that the symptom was most severe at $1.20x10^3$cfu/ml while it was least severe at $5x10^2$cfu/ml of *Escherichia coli*. Symptom was most severe at $1.48x10^3$cfu/ml and least severe at $6.4x10^2$cfu/ml of *Staphylococcus aureus* while *Pseudomonas aeruginosa* was most severe at $1.20x10^3$cfu/ml and it was least effective at $5.0x10^2$cfu/ml. The physical observation of the albino rats showed that healthy rats had formed stool (solid, round, short rods), they were eating well, and they were active and had normal skin. It was also observed that the rats became healthy after some time without treatment. This result is shown in table 3, 4, 5, 6.

Table 1. *Zone of inhibition of the extract on the organisms at concentration of 150mg/ml.*

Test organisms	Zone of inhibition (mm)
Klebsiella pneumoniae	-
Pseudomonas aeruginosa	8.0
Steptococcus pyogenes	-
Escherichia coli	8.0
Staphylococcus aureus	6.0
Enterobacter aerogenes	-

Table 2. *Minimum inhibitory concentrations (MIC).*

Test organisms	Minimum Inhibitory Concentration (MIC) (mg/ml)
Klebsiella pneumonia	-
Pseudomonas aeruginosa	50
Steptococcus pyogenes	-
Escherichia coli	50
Staphylococcus aureus	100
Enterobacter aerogenes	-

Table 3. *Colony forming units (cfu/ml) of the bacteria used (infectivity dose).*

Organisms	Cfu/ml	Days infection set in
Escherichia coil	$1.20x10^3$	3
Staphylococcus aureus	$1.48x10^3$	2
Pseudomonas aeruginosa	$1.80x10^3$	2

Table 4. *Symptoms observed in albino rats when infected with Escherichia coli.*

Escherichia coli					
Infectivity dose	**24 hrs**	**48hrs**	**72hrs**	**96hrs**	**120hrs**
$1.2x10^3$	FS, A, EW	US, W, LA	US, W, LA	FS, A, LA	FS, A, EW
$8.2x10^2$	FS, A, EW	FS, A, EW	US, LA, W	FS, A, LA	FS, A, EW
$5.0x10^2$	FS, A, EW	FS, A, EW	FS, A, EW	FS, A, EW	FS, A, EW

Table 5. *Symptoms observed in albino rats when infected with Staphylococcus aureus.*

Staphylococcus aureus					
Infectivity dose	**24 hrs**	**48hrs**	**72hrs**	**96hrs**	**120hrs**
$1.48x10^3$	US, W, LA, FD	US, W, LA, FD	FS, W, LA, FD	FS, A, EW, FD	FS, A, EW, NS
$9.2x10^2$	FS, A, EW, NS	FS, A, EW, FD	US, LA, W, FD	US, W, LA, NS	FS, A, EW, NS
$6.4x10^2$	FS, A, EW, NS	FS, A, EW, NS	FS, A, EW, FD	FS, A, LA, FD	FS, A, EW, NS

Table 6. *Symptoms observed in albino rats when infected with Pseudomonas aeruginosa.*

Pseudomonas aeruginosa					
Infectivity dose	**24 hrs**	**48hrs**	**72hrs**	**96hrs**	**120hrs**
120	FS, EW, A	FS, LA, W	US, LA, W	US, LA, W	US, LA, W
82	FS, EW, A	FS, EW, A	FS, LA, W	US, LA, W	US, LA, W
50	FS, EW, A	FS, EW, A	FS, EW, A	FS, EW, A	FS, LA, W

KEYS: FS- Formed stool; US- Unformed stool; A- Active; W- Weak; EW- Eaten well; LA- Loss of appetite; NS- Normal skin; FD- Fur dropping

3.4. Effect of Treatment on the Rats

During treatment, it was observed that the rats responded to treatment positively. Rats infected with *Escherichia coli* had consistent stool, they became active and their feeding rate was restored. Positive response was also observed in rats infected with *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Table 7 indicates the trend of response of the infected albino rats to treatment with ethanolic leaf extract of *Carica papaya.*

Table 7. *Response of the infected albino rats to ethanolic leaf extract of Carica papaya.*

Organisms	Duration of treatment				
	24 hrs	48hrs	72hrs	96hrs	120hrs
Escherichia coli	US, W, LA	US, W, LA	FS, A, EW	FS, A, EW	FS, A, EW
Staphylococcus aureus	US, W, LA, FD	US, W, EW, FD	FS, W, LA, FD	FS, A, EW, NS	FS, A, EW, NS
Pseudomonas aeruginosa	US, W, LA	FS, W, LA	FS, W, LA	FS, A, EW	FS, A, EW

KEYS: FS- Formed stool; US- Unformed stool; A- Active; W- Weak; EW- Eaten well; LA- Loss of appetite; NS- Normal skin; FD- Fur dropping

3.5. Haematology Test

The result of the haematology test showed variation between the control, infected and treated albino rats. There was decrease in Erythrocyte sedimentation rate, Packed cell volume, Haemoglobin estimation, Red blood cell count and Lymphocyte during infection while there was increase in White blood cell count, Neutrophil, Eosinophil, Monocyte and Basophil during infection. The haematological result obtained from treated rats is close to that of the control. This result is shown in table 8, 9, 10.

Table 8. *Haematology test on albino rats infected with Escherichia coli.*

	ESR	PCV	HB	WBC	RBC	LYMP	NEUT	EOS	MONO	BAS
A	1.0	36	12.67	9.0	6.20	75	20	3	2	0
B	1.0	37	14.0	8.0	6.2	76	18	3	2	1
C	1.3	40	18.6	7.6	6.8	79	18	2	1	0

Table 9. *Haematology test on albino rats infected with Staphylococcus aureus.*

	ESR	PCV	HB	WBC	RBC	LYMP	NEUT	EOS	MONO	BAS
A	1.1	32	10.67	12.5	5.85	65	27	5	2	1
B	1.2	38	13.4	8.5	6.50	77	17	4	2	0
C	1.3	40	18.6	7.6	6.8	79	18	2	1	0

Table 10. *Haematology test on albino rats infected with Pseudomonas aeruginosa.*

	ESR	PCV	HB	WBC	RBC	LYMP	NEUT	EOS	MONO	BAS
A	1.0	30	10.00	13.8	5.70	64	28	5	2	1
B	3.0	38	16.0	15.3	6.10	78	17	3	1	1
C	1.3	40	18.6	7.6	6.8	79	18	2	1	0

KEYS: A-Infected rat; B-Rat treated with ethanolic leaf extract of *Carica papaya*; C-Healthy rat/ control; ESR- Erythrocyte sedimentation rate; PCV-Packed cell volume; HB-Haemoglobin estimation; RBC- Red blood cell count; WBC- White blood cellcount; LYMP- Lymphocyte; NEUT-Neutrophil; EOS-Eosinophil; MONO- Monocyte; BAS- Basophil.

3.6. Histopathological Analysis

This was carried out on the vital organs of the albino rats. Intestine was analyzed for *Escherichia coli* while heart was analyzed for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The comparism between the infected and treated rats as well as control for each of the rats infected with *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* is shown in Figure 1-9.

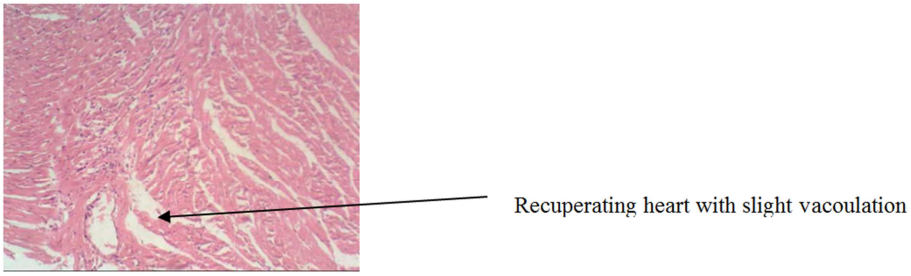

Figure 1. *Infected and treated.*

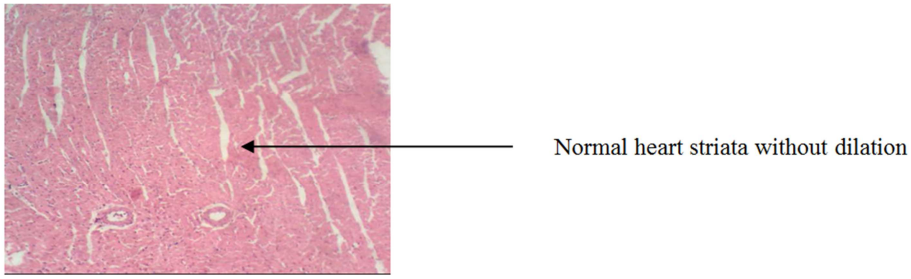

Figure 2. *Control group.*

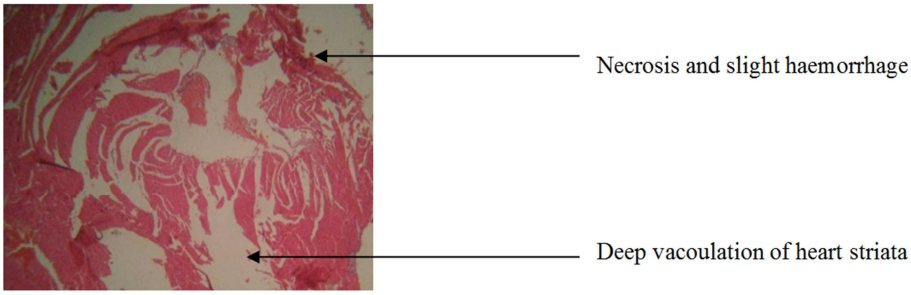

Figure 3. *Infected and not treated.*

Figure 1, 2 and 3: photomicrograph of the heart of albino rat during infection, after treatment (*Staphylococcus aureus*) and control.

Figure 4. *Infected and not treated rat.*

Figure 5. *Control group.*

Figure 6. *Infected and treated.*

Figure 4, 5 and 6: Photomicrograph of heart of rat during infection and after treatment (*Pseudomonas aeruginosa*) and control.

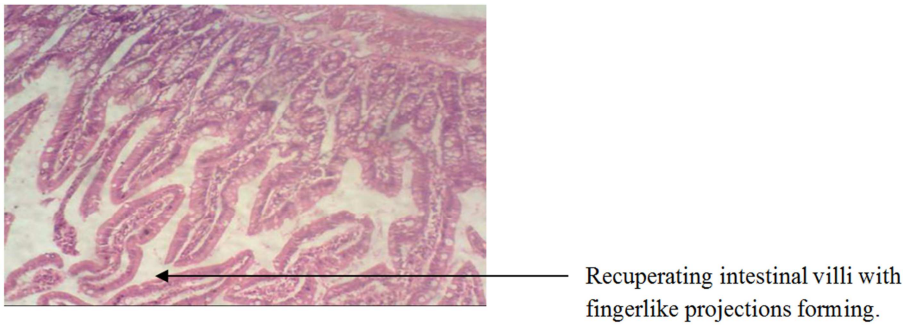

Figure 7. *Infected and treated.*

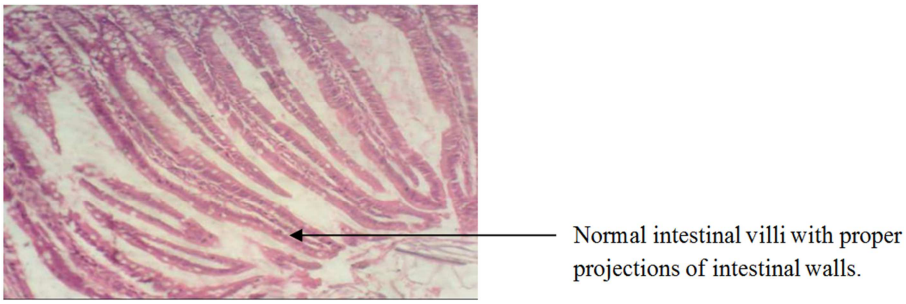

Figure 8. *Control.*

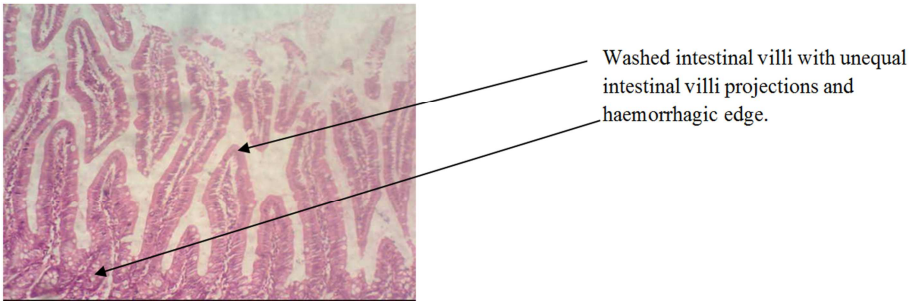

Figure 9. *Infected and not treated.*

Figures 7, 8, and 9: showing the small intestine of rat during infection, after treatment (*Escherichia coli*) and control.

4. Discussion

Medicinal plants are of great importance to the health of individuals. They constitute an effective source of both traditional and modern medicine and about 80% of the rural population depend on it as primary health care [14]. The phytochemical screening of the leaf extract showed that cardenolides and saponins are present in the extract. These bioactive compounds may be responsible for the antibacterial properties of the ethanolic leaf extract. The plant extract had no antibacterial effect on *Klebsiella pneumonia, Enterobacter aerogenes* and *Streptococcus pyogenes*. This may be because the organisms are not sensitive to the bioactive components in the extract and also due to the high formidable permeability of gram negative bacteria. Antimicrobial agents make contact with the cell envelope during this process; the rate of penetration may be reduced. The structural difference may play a key role in the susceptibility of the test organisms [15].

Some of the organisms were inhibited in all the concentration of the extract while some were only inhibited at higher concentrations, but the higher the concentration the greater the zone of inhibition. Ekwenye and Elegalam [16] and Azu and Onyeagha [17] reported that the efficacy of most plant extracts is concentration dependent. This may be because the concentration of the bioactive components is small in lower concentrations. The susceptibility of the microorganisms to the plant extract may be as a result of the structure of their cell walls.

The animal bioassay showed that there was decrease in the average weight of the rats during infection. Animals infected with *Escherichia coli* and *Staphylococcus aureus* showed significant weight loss during infection. This may be as a result of loss of appetite, diarrhea and dehydration. In this case, they lose a lot of fluid and take in little or no food and water to restore the lost nutrients, this weakens the animals and lead to loss of weight. During the determination of infectivity dose, it was observed that the animals became healthy after about 3-4 days of infection without they were treated. This may be because the

infections are self limiting.

Haematological study carried out on the blood showed that there was decrease in the percentage of Erythrocyte sedimentation rate, packed cell volume, Haemoglobin estimation, Red blood cell count and Lymphocyte during infection. This may be as a result of suppression in the immune system. Reduction in PCV is an indication of infection and it could be a sign of anaemia. Increase in White blood cell count, Neutrophil, Eosinophil, Monocyte and Basophil during infection may be because they help to fight against infections. Therefore, their population increased during infection in order to combat and evade the microorganisms. Increased white blood cells fight and combat the microbes.

In the histopathological analysis, the photomicrograph of the heart of rat infected with *Pseudomonas aeruginosa* showed necrotic edge and divergence of heart striata. This may be as a result of distortion of the heart by the microorganism. The treated rat showed recuperating heart with unequally distributed striata. This indicates that the ethanolic leaf extract was able to heal the heart although not as perfect as the control. The deep vacoulation of the heart stiata observed in the heart of frat infected with *Staphylococcus aureus* could be as a result of the presence of the microorganism. The treated heart had recuperating heart with slight vacoulation while the control had normal heart striata with no dilation. Intestine of rat infected with *Escherichia coli* had washed intestinal villi projections and haemorrhagic edge while the control had normal intestinal villi with normal projections of intestinal walls. The distortion observed in the intestine of infected rat may be as a result of the release of large amount of water or continuous washing of the intestine with large amount of water. The treated rat had recuperating intestinal villi with finger- like projections. Finally, it was observed that the rats had high tolerance to the plant extract because the extract posed little negative effect on the organs of the mammals.

5. Conclusion

Conclusively, Carica papaya ethanolic leaf extract had antibacterial effect against Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus while Klebsilella pneumoniae, Streptococcus pyogenes and Enterobacter aerogenes. Zones of inhibition increased with increase in concentration of extract, therefore the higher the concentration, the higher the zone of inhibition. However, further purification of bioactive compounds present in Carica papaya leaf extract is necessary, as this may produce greater result and may be used industrially for synthetic production of drugs. The result from the in-vivo analysis revealed that the extract is relatively safe for consumption.

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