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In vivo Evaluation of Microorganisms Isolated from Peels of Selected Carbohydrate Rich Tubers

A. O. Momoh[1*] and O. S. Fadare[1]

[1]Department of Biological Sciences, Microbiology Unit, Elizade University, Ilara Mokin, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author AOM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Author OSF managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To detect the effect of microbes isolated from peels of Carbohydrate rich tubers on Albino rats.
Study Design: The study was a comparative and descriptive study. Cassava and cocoyam peels were bought from Obanla market, Federal University of Technology, Akure. Those samples were put into a sterile polythene bag and transported to microbiology laboratory for microbiological analysis.
Place and Duration of Study: Microbiology Laboratory, Elizade University, Department of Animal Production and Health Research Laboratory, Federal University of Technology, Akure, Ondo State. The study was performed between September, 2015 and March, 2016
Methodology: Two gram of the samples was grounded in a mortar with pestle. The serial dilution method was aseptically used to reduce the microbial load present in the collected samples. Nutrient agar and potato dextrose agar were used to isolate the bacteria and fungi respectively from the samples under aseptic condition. Biochemical test and sugar fermentation were carried out on the bacterial isolates after pure culture was obtained.

**Corresponding author: E-mail: abdul.momoh@elizadeuniversity.edu.ng;*

Results: The isolated bacteria were *Streptococcus faecalis, Corynebacterium fascians, Micrococcus luteus, Alcaligenes faecalis* and *Aeromonas hydrophila*. Fungal isolates were identified using lactophenol cotton blue. Fungi isolated includes: *Articulospora inflata, Aspergillus fumigatus, Aspergillus niger, Geotrichum albidum, Torula herbarum and Halosporangium panum.* All the bacteria isolates were used to infect Albino rat. The uninfected rats were used as control. The histopathological and haematological analysis carried out on liver and intestine of the albino rats showed that the microorganisms caused a pathological change that ranges from necrosis of the liver hepatocytes, hepatocellular drainage and hemorrhage to vacuolation and inflammatory cell infiltrations of the intestinal wall.

Conclusion: This study has shown that cassava and cocoyam peels can act as vehicles for disseminations of pathogenic microorganisms and should therefore be properly disposed.

Keywords: Intestine; liver; microorganisms; pathogens; tuber peels.

1. INTRODUCTION

In the agricultural sector, tonnes of waste are generated yearly and they are ignored causing nuisance to the environment thereby resulting to environmental pollution and aesthetic irritant [1]. These wastes pose a disposal problem and tend to be more problematic with increase in industrial production without converting the waste to useful forms. Limitations to the use of crop waste include; low digestibility, low protein content and excessive crude fiber [2]. Fermentation has been identified as a technique that is inexpensive for the detoxification and increase in protein quality of some of such products [3].

Root and tuber crops are second only in importance to cereals as a global source of carbohydrates [4]. They also provide some minerals and essential vitamins, although a proportion of the minerals and vitamins may be lost during processing as, for example, in the case of cassava [5]. The quantity and quality of the protein in starchy staples varies and relatively low on a fresh weight basis but compare favourably with some cereals on a dry weight basis. In most traditional diets vegetable soups, meat, groundnuts, grain legumes and fish are good sources of protein and are frequently used to supplement root crops and compensate for their protein deficiencies. In some parts of Africa the diet is supplemented with the tender leaves of sweet potato, cassava and cocoyam which are rich sources of protein, minerals and vitamins [6].

In tropical Africa root and tuber crops still constitute important, often major, components in traditional diets. In rural areas there is usually an adequate supply to meet any local demand from the surplus production of subsistence farmers and by local trade [7]. The demand for food in large urban areas has increased, and continues to increase due to the large population migration from the rural areas, which has continued for thirty years and shows no signs of abating [8]. The migrating rural population takes with it its traditional eating habits, particularly until it becomes urbanized, despite the opportunities for diversification of eating habits with the choice of food available in the towns [9]. Root crops, particularly cassava, remain in demand, but this demand is often unsatisfied because of the inherent limitations of the traditional production systems which impose serious constraints in marketing and processing [10]. The general pattern of the supply of root crops from the surpluses of subsistence farming leads to high marketing costs and high consumer prices. As a consequence, in urban communities the consumption of root crops tends to be replaced by imported cereals, rice and wheat flour [11]. There is need to assess the microbiological impact of these wastes in our environment on animals that feed on them.

2. MATERIALS AND METHODS

2.1 Required Materials and Apparatus

The following materials and apparatus were used: cassava peel, cocoyam peel, petri dishes, inoculating loop, beaker, syringe and needle, polythene bag, cotton wool, microscope, measuring cylinder, autoclave, inoculating needle, nutrient agar, potato dextrose agar, slides, cover slips, conical flask, aluminum foil, and distilled water.

2.2 Collection of Sample

The samples used (cassava peel and cocoyam peel) were bought from Obanla, University of Technology, Akure. Each of the samples was separately placed in sterile polythene bag and taken to the laboratory for microbial analysis.

2.3 Sterilization of Glass Wares and Chemical Reagents

All the glass wares such as Petri-dishes, Durham tubes, test-tubes, McCartney bottles, conical flasks, and metal apparatus such as spatula were washed with detergents, rinsed with clean water, dried and sterilized at hot oven at 160^{o}C for 1hour. Alcohol (95%) was used to sterilize the bench tops, plastic containers, laboratory bench and inoculating chamber before used. Media used were prepared and sterilized according to manufacturer's specifications. Chemical reagents used for chemical analysis and biochemical characterization tests were prepared according to the requirements each analysis.

2.4 Media Preparation

The general purpose media, nutrient agar was prepared according to manufacturer's specification, that is 28 g of nutrient agar was dissolved in 1 litre of distilled water and 39 g of Potato Dextrose Agar was also dissolved in 1 litre of distilled water and 0.1 ml of the inoculums were introduced into the Petri-dishes before 20 ml of the media were poured into the plates in triplicates.

2.5 Methods of Identification of Bacteria Isolates

Characterization of bacterial isolates was based on standard microbiological techniques described by Fawole and Oso, [12]. Gram staining, morphological and cultural characteristics were carried out with various biochemical tests which include: catalase test, coagulase test, oxidase test, motility test, and starch hydrolysis, and sugar fermentation [13].

2.6 Antibiotics Susceptibility Test of the Bacteria Isolates

One ml of 24 hr old young broth culture was dispensed into 9 sterile Petri dishes. It was mixed with sterile molten nutrient agar, allowed to cool and a sensitivity disc was placed firmly on the agar with sterilized forceps to ensure complete contact with the agar. These steps were repeated for every test bacterium. The plates were incubated for 24 hours. The susceptibility of each isolate to each antibiotic as indicated by clear zones of inhibition was measured in mm using metre rule [14].

2.7 Haematological Analysis

Young adult albino rats (3 weeks old) were obtained and allowed to acclimatize for one week before infecting them with each type of bacteria in triplicates after determining the infectivity dose using the method described by Momoh, [15]. Rats that were not infected constituted the control. At the end of the experiment, blood samples were collected into separate EDTA bottles for analysis at the Federal University of Technology, Akure, (Department of Animal Production and Health Research Laboratory). Full blood cell and WBC Differential counts were carried out using standard methods described below:

2.7.1 Erythrocyte sedimentation rate (ESR)

A Wintrobe tube was filled to the top 0 mark and one end of it blocked with plasticine. It was placed undisturbed in an upright position for 60minutes. The distance of the fall of red cells from 0 marks was read and expressed in mm/hr as the ESR.

2.7.2 Packed cell volume (PCV)

Each blood sample collected into the anticoagulant bottle (EDTA) was mixed and a capillary tube (CT) was filled up to 75% (3/4) of its length and placed in the micro-haematocrit centrifuge with the sealant at the outer end and centrifuged at 12,000 rpm for 5 minutes. The result of the ratio of the packed cells to the separated plasma was read as a percentage of packed red cells to total volume of whole blood using a haematocrit reader.

2.7.3 Red blood cell count (RBC)

Every blood sample was diluted (1:200) using sodium metabisulphite and mixed properly. The diluted blood (0.02 ml) was pipette into 4 ml of diluting fluid in a bijou bottle and washed thoroughly by alternately drawing up and expelling the diluting fluid. A fine Pasteur pipette was used to fill a blood cell counting chamber (haemocytometer) and counted using a counter under a light microscope at × 40 magnification objectives.

2.7.4 White blood cell count (WBC)

The blood was first diluted in ratio 1:20 and 0.05 ml of the blood was pipetted into 0.95 ml of diluting fluid. A little portion was charged into the

counting chamber and observed using ×10 objective to count the white cells/cubic mm.

2.7.5 Haemoglobin (Hb)

Using mouthpiece, sucker and a 0.02 ml pipette, blood was withdrawn and expelled into 4 ml Drabkin's solution in a tube. The tube was stoppered, mixed and allowed to stand for 5 minutes for full colour development. A standard blood sample of known haemoglobin concentration was prepared. Using a green (624) filter, the calorimeter was set to zero using plain Drabkin's solution as a blank. The readings of the sample and the standard were taken and the result calculated as follows:

Sample haemoglobin concentration = (Reading of test × standard haemoglobin concentration / Reading of standard)

2.7.6 White blood cell differential (WBC differential)

These are divided into granulocytes and agranulocytes. The granulocytes are further divided into three which are neutrophils, eosinophils and basophils. These were counted after staining with Giemsa stain and their numbers recorded. The agranulocyte are equally further divided into two, which are lymphocytes and monocytes.

2.8 Histopathological Studies

Histopathological examination was carried out on the albino rats used for haematological analysis. The organs (liver) were removed, grossly examined and stained with haematoxylin-eosin before examining under the light microscope to know if the treatment has any effect on them. The organs were compared with that of the control rats. The histological processing was carried out and interpreted at the Animal Production and Health Laboratory, Federal University of Technology, Akure, Ondo State. Histopathological tests were carried out on the organs of the laboratory animals as follows: Organs of the animals were collected and fixed in 10% formalin to prevent decay. They were dehydrated in different percentage (50%, 70%, 80% and 100%) of alcohol 1 ½ hours each. After dehydration they were cleared with 100% xylene and left for 2 hours to remove any remnant alcohol and impregnated in liquid wax for 2 hours for embedding. The embedded organs were sectioned using microtome and were stained with haematoxylin-eosin [16]. Excess stain was removed with tap water. After clearing in xylene, Canada balsam was added and cover slips placed on the slides. The preparations were left in the oven at 40℃ and then placed under the microscope with a digital camera connected to a computer system to be examined by an expert and take the photographs.

2.9 Statistical Analysis of Result

Numerical values obtained were subjected to descriptive one way analysis of variance, SPSS version 16 Microsoft Windows 7 and Duncan Multiple Range Test were used.

3. RESULTS

The cultural characterization of the isolates was physically observed in which the isolates showed differences in their colony morphology (i.e. colour, shape, edge, elevation and surface)

The biochemical analysis and sugar fermentation tests (Table 1) were carried out on the isolated microorganisms where *Corynebacterium fascians* and *Micrococcun luteus* were both catalase positive. The result of the isolation and identification process from the cassava and cocoyam peels showed that a total of six bacteria were isolated. These are; *Corynebacterium fascians, Micrococcus luteus, Aeromonas hydrophila, Aerococcus viridians, Streptococcus faecalis and Alcaligenes faecalis.* The most prominent bacteria were; *Corynebacterium fascians, Micrococcus luteus* and *Aeromonas hydrophila* because they were all found in cassava and cocoyam peels. *Aerococcus viridians* was found in cassava peel and both *Streptococcus faecalis and Alcaligenes faecalis* were found in cocoyam peel.

Result of antibiotics susceptibility of isolated microorganisms from cassava and cocoyam peels showed that all the isolated organisms have the antibiotics susceptibility value range from 0 to 10. The following fungi were commonly found in cassava and cocoyam peels used in this project: *Articulospora inflata*, *Aspergillus fumigatus*, *Geotrichum albidum* and *Aspergillus sp*. *Torula herbarum* was isolated from cocoyam peel while *Halosporangium panum wa*s found in the cassava peel used for this experiment.

Table 1. Biochemical analysis and sugar fermentation of the bacteria isolated from cassava and cocoyam peels

Isolates	Biochemical test							Sugar fermentation				
Cassava	**Gram stain**	**Catalase**	**Oxidase**	**Starch Hydrolysis**	**Motility**	**Spore**	**Coagulase**	**Glucose**	**Galactose**	**Fructose**	**Maltose**	**Sucrose**
1	+	+	_	_	_	_	_	AG	AG	_	AG	AG
2	_	_	_	_	_	_	_	A	_	A	_	AG
3	_	+	_	_	_	_	_	A	_	A	A	A
4	+	+	_	_	_	_	_	A	A	_	A	A
Cocoyam	**Gram stain**	**Catalase**	**Oxidase**	**Starch Hydrolysis**	**Motility**	**Spore**	**Coagulase**	**Glucose**	**Galactose**	**Fructose**	**Maltose**	**Sucrose**
1	+	+	_	_	_	_	_	AG	AG	_	AG	AG
2	+	+	_	_	_	_	_	A	A	_	A	A
3	_	_	_	_	_	_	_	A	_	A	_	AG
4	+	_	_	_	_	_	_	A	A	_	_	A
5	_	_	+	+	+	_	_	A	A	A	_	_

Key= A: Acid production, AG: Acid and gas production. 1-4;1-5:Specimen Number

Table 2. The probable microorganism isolated from cassava and cocoyam peels based on the morphological and biochemical characteristics

Sample	Specimen number	Probable microorganisms
Cassava	1	*Corynebacterium fascians*
	2	*Aeromonas hydrphila*
	3	*Aeromonas viridians*
	4	*Micrococcus luteus*
Cocoyam	1	*Corynebacterium fascians*
	2	*Micrococcus luteus*
	3	*Aeromonas hydrophila*
	4	*Streptococcus faecalis*
	5	*Alcaligenes faecalis*

Table 3. Antibiotics susceptibility of isolated microorganisms from cassava and cocoyam peels

Isolates	Zone of inhibition (mm)									
	CHL	CRO	GEM	PFX	COT	CPX	ERY	AMX	OFL	AUG
C. fascians	5	10	10	9	10	7	8	8	10	10
M. luteus	10	9	8	10	10	8	10	9	10	8
S. faecalis	9	8	10	10	10	9	8	9	10	10
A. hydophilis	8	10	9	10	8	10	5	0	10	0
A. faecalis	5	6	0	0	8	5	5	5	5	0
A. viridans	6	5	5	6	5	4	5	4	4	0

KEY= CHL: Chloramphenicol (30 µg), CRO: Cefriazole (30 µg), PFX: Pefloxacin (5 µg), COT: Cotrimazole (30 µg) CPX: Ciprofloxacin (10 µg), ERY: Erythromycin (5 µg), AMX: Amoxillin (25 µg), OFL: Ofloxacin (5 µg), AUG: Augmentin (30 µg)

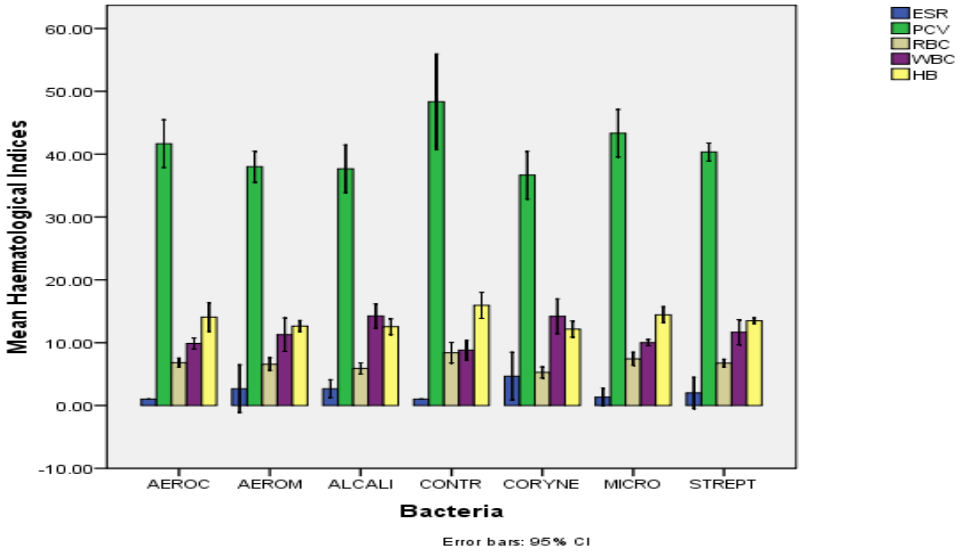


Fig. 1. Heamatological indices of infected albino rats

Key: AEROC : Aerococcus viridians, AEROM: Aeromonas hydrophila, ALCALI: Alicaligenes faecalis, CONTR: Control, CORYNE: Corynebacterium fascians, MICRO: Micrococcus luteus, STREPT: Streptococcus faecalis; ESR: Erythrocyte Sedimentation Rate, PCV: Packed cell volume, RBC: Red Blood Cell count, WBC: White Blood Cell count, HB: Haemoglobin Concentration

The result of the haematological analysis carried out on the blood of the infected albino rats showed that the infections caused a reduction in the PCV of the rats. ESR values were generally high in all the infected animals with the different isolates. Comparatively, the PCV of the control

was high, almost at 50% followed by that of the rats infected with *Micrococcus luteus* and *Aerococcus viridians*. This result is shown in Fig. 1.

The result of WBC Differential count carried out on the blood of the infected albino rats showed that only the set of rats used as control has the highest value of Lymphocytes, *Aerococcus viridians* and *Micrococcus luteus* have the same value of Lymphocytes while the lowest value was observed in animals infected with *Corynebacterium fascians.* The animals infected with *Corynebacterium fascians* that have the lowest value of the Lymphocytes, possess a highest value in Monophils while the set of albino rats used as control has the least value. Therefore, the rats infected with *Corynebacterium fascians* have highest value in Neutrophils and Monophils but lowest value in Lymphocytes (Fig. 2).

The histopathology of the intestine and the liver showed high cell infiltrations. The major negative pathological sign which was seen in the heart and intestine was in treatment E were there was severe haemorrhage.

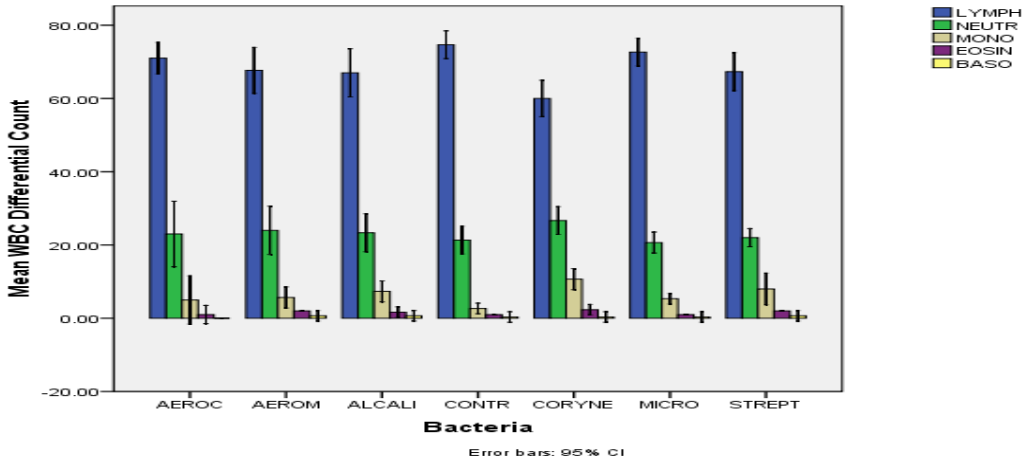
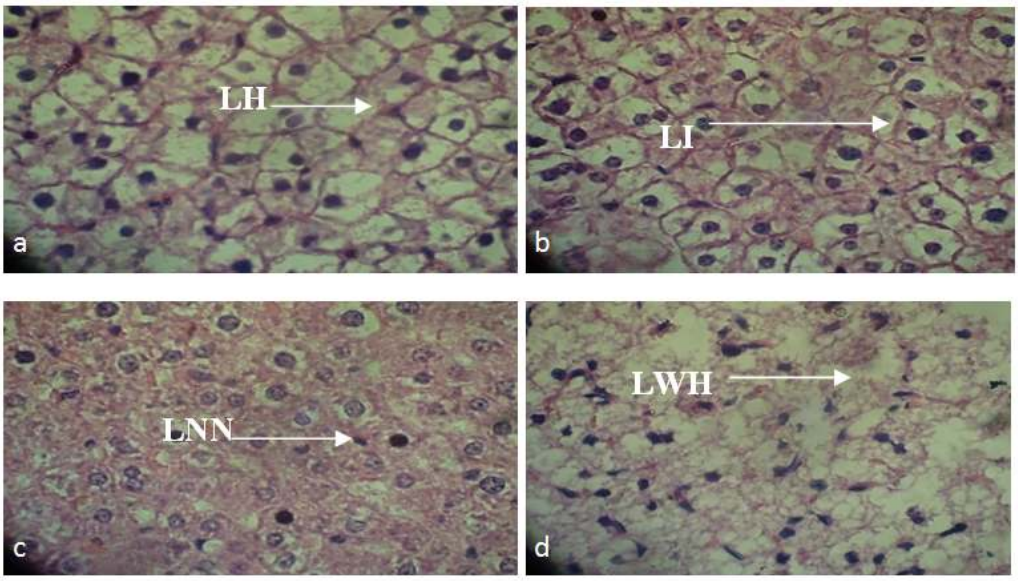


Fig. 2. Mean WBC differential count of the infected albino rat

Key: AEROC: Aerococcus viridians, AEROM: Aeromonas hydrophila, ALCALI: Alicaligenes faecalis, CONTR : Control, CORYNE: Corynebacterium fascians, MICRO: Micrococcus luteus, STREPT: Streptococcus faecalis; Lymph: Lymphocytes, Neutr: Neutrophils, Mono: Monophils, Eosin: Eosinophils, Baso: Basophils.



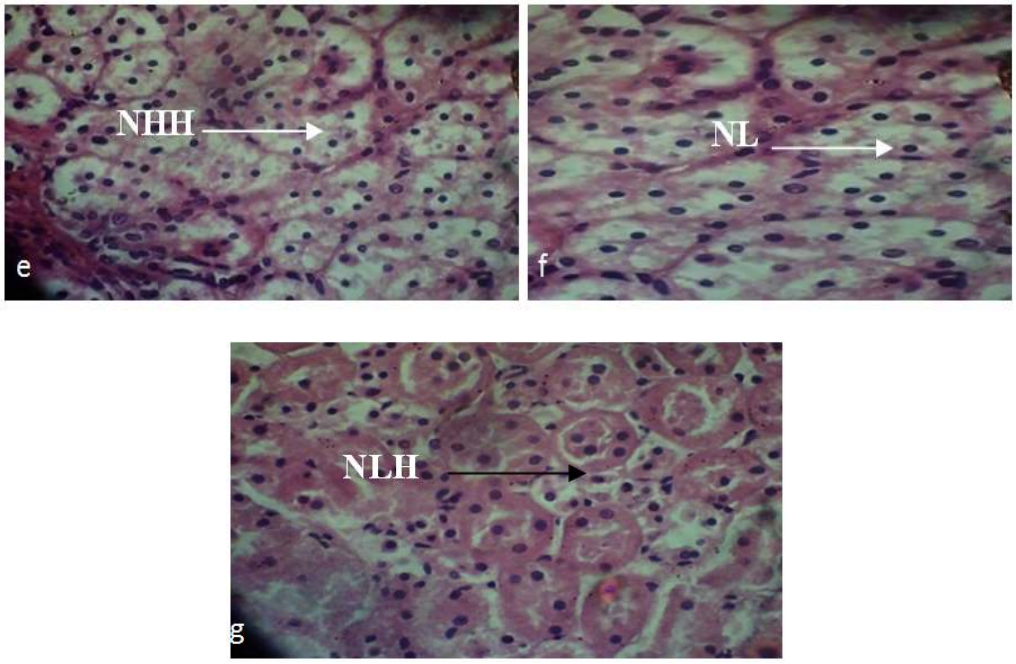


Plate 1a - 1g. Histopathology of liver hepatocytes of albino rats infected with bacteria.
a.) *Corynebacterium fascians,* b.) *Micrococcus luteus* c.) *Aeromonas hydrophila*
d.) *Aerococcus viridians*, e.) *Streptococcus faecalis,* f.) *Alcaligenes faecalis* g.) Control
Key: LH: Liver with haemorrhage, LI: Liver with infiltration, LNN: Liver with necrotized nucleus, LNHI: Liver with washed hepatocytes and inflammatory cell, NHH: Normal liver hepatocytes with slight haemorrhage, NL: Normal liver, NHL: Well formed normal liver hepatocytes (control)

4. DISCUSSION

The result shows that a total of six bacteria were isolated. These are; *Corynebacterium fascians, Micrococcus luteus, Aeromonas hydrophila, Aerococcus viridians, Streptococcus faecalis and Alcaligenes faecalis.* Though some of these bacteria are free-living microorganisms, others are pathogenic. According to Prescott et al*.* [17], the fact that some free-living bacteria are present in a habitat is a pointer to the fact that pathogenic bacteria could also colonize such habitat.

The fungal isolates were: *Articulospora inflata, Aspergillus fumigatus, Aspergillus niger, Geotrichum albidum Torula herbarum and Halosporangium panum.* The fungi isolated are generally saprophytic decomposer. According to Brooks et al*.* [18] *Aspergillus* species are majorly opportunistic microorganisms except *Aspergillus flavus* which produces aflatoxin in foods. Therefore, the presence of these isolated microorganisms calls for caution on our methods of disposal of food or agricultural wastes.

Most of the standard antibiotics used on the bacteria isolates were effective on them. Ceftriazole and gentamycin were most effective on all the isolates. This result is in agreement with the work of Egbunike et al. [3], who used those antibiotic discs and found them effective on most of the subject bacteria.

The result obtained in this work also shows that the infection of the rats with these microorganisms cause pathological changes in the liver of albino rats. These effects include necrosis, haemorrhage, inflammatory cell, infiltration and washing of hepatocytes. These pathological changes are clear indication of the pathogenicity of these organisms when compared with control. These features are similar to the result contained by Momoh, [15] who infected albino rat with some of the microorganisms and got the same features.

According to Prescott et al. [17] these features are usually caused by the performance of these microorganisms when they enter the blood stream and pass through the liver during systemic circulation. The histopathology of the intestine shows that the microorganisms caused various pathological changes that range from

distribution of blood capillaries in the intestinal wall to central bilation of intestinal mucosa. These features according to Brooks et al. [18] are caused by bacteria that have the ability to penetrate mucosa through the intestinal wall. They often lead to production of ulcer in the intestinal wall of man and animal when fully established.

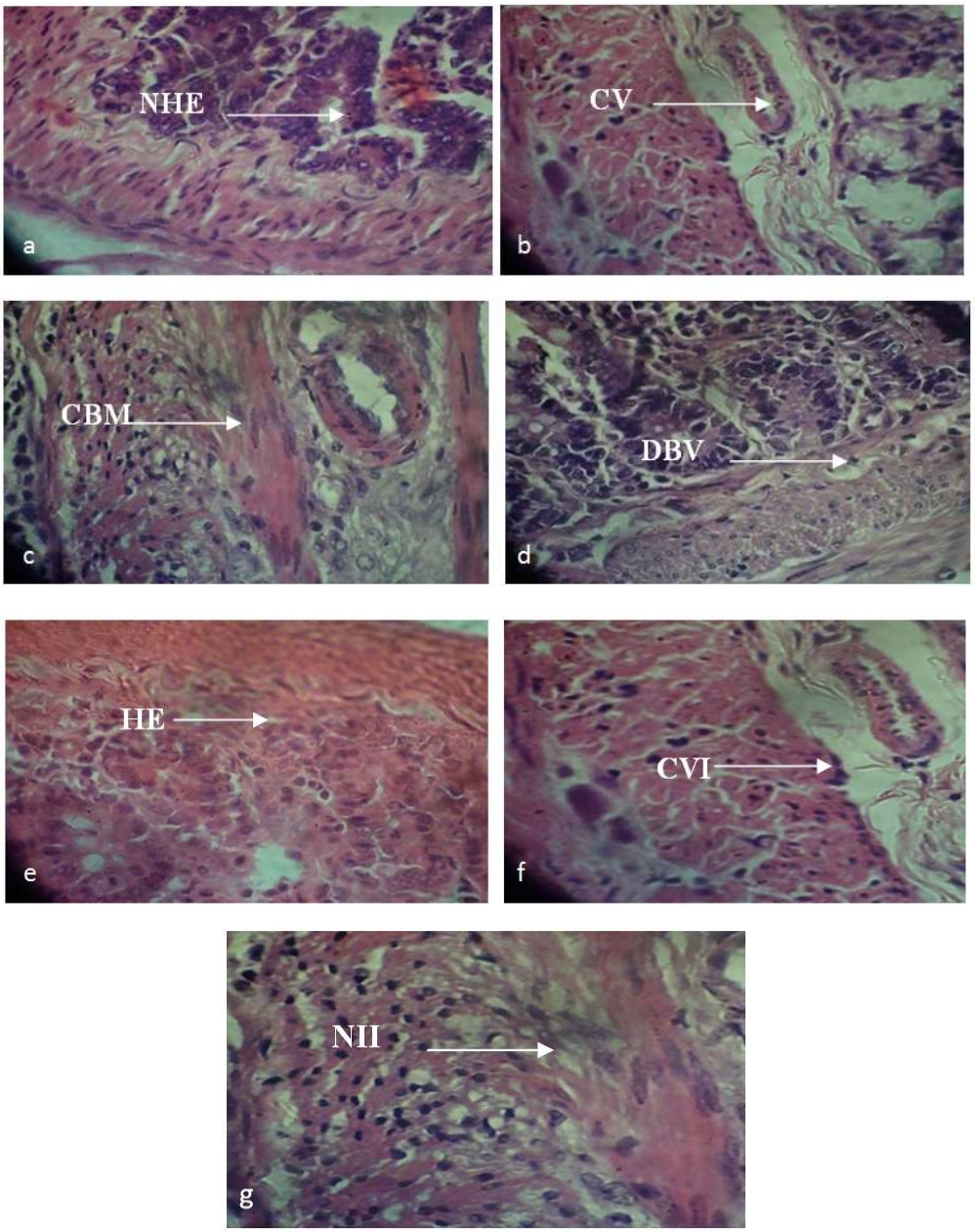


Plate 2a-2g. Histopathology of intestine of albino rats infected with bacteria

a. Corynebacterium fascians, b. Micrococcus luteus c. Aeromonas hydrophila d. Aerococcus viridians, e. Streptococcus faecalis, f. Alcaligenes faecalis g. Control. KEY: NHE: Intestine with necrotized heamorrhage and eroded intestinal wall; CV: Intestine with control vacuolation; CBM: Intestine with central bilation of intestinal mucosa; DBV: Intestine wall deficient of blood vessels; HE: Intestine with haemorrhage end; CVI: Intestine with central vacuolation and inflammatory cell; NII: Normal intestine with cell infiltration

5. CONCLUSION

This work has shown that cassava and cocoyam peels house variety of microorganisms and should be disposed properly. The isolated microorganisms from cassava and cocoyam peels used in this study caused the pathological changes in the infected albino rat. It is therefore logical to conclude that, these organisms are responsible for these pathological changes and could possibly cause similar pathological changes in man if ingested. Asides the pathological changes these microbes are able to cause in animals and man, they are also useful in the degradation of plant wastes and invariably important in prevention of waste pollution as they help in the decomposition of the waste within a shorter period.

Further work on the involvement of those isolates in degradation as well as in fermentation of those wastes for possible use in feeding animals such as goats and cows should be assessed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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