International Scholars Journals

International Journal of Urology and Nephrology ISSN 2756-3855 Vol. 8 (4), pp. 001-006, April, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

Full Length Research Paper

Toxicological renal effects of *Bridelia ferruginea*-treated wastewater in rats

Kolawole O. M.[1], Olayode J. A.[2], Oyewo O. O.[2], Adegboye A. A[1] and Kolawole C. F.[2]

[1]Department of Microbiology, Faculty of Science University of Ilorin, Nigeria.

[2]Anatomy Department, Ladoke Akintola University of Technology Ogbomosho, Oyo State, Nigeria.

Accepted 16 February, 2020

The effect of wastewater fed to rats following treatment with a 2.5% (w/v) methanolic crude extract of *Bridelia ferruginea* for different times (0 to 96 h) was investigated. Kidneys of rats fed with the treated wastewater was subjected to histological examination and compared to negative (rats given wastewater treated with methanol alone), positive (rats given untreated wastewater alone) and standard (rats given sterile distilled water alone) control groups. Histopathological findings revealed extensive damage in the tissues of rats assigned to the negative, positive and *B. ferruginea* extract-treated groups. However, no damage was observed in the tissues of the standard control group. The potential efficacy and the toxicological renal effects of *B. ferruginea* extract-treated wastewater is discussed.

Key words: *Bridelia ferruginea*, wastewater, bacterial count, kidney.

INTRODUCTION

Domestic wastewater can be defined as the wastewater of communities carried from residences, commercial buildings and industrial plants. It varies from hour to hour, both in quantity and in chemical composition (Hammer, 1985). Domestic wastewater, which usually has an almost neutral pH, may be categorized by its dissolved oxygen content as fresh, stale or septic (Rowe, 1994). Indeed, polluted waters are sources of waterborne diseases that are responsible for many deaths in developing countries (Bashir and Adebayo, 2003). Water contamination is one of the most important environmental problems faced by third world countries. The accumulation of urban wastes and problems relating to their disposal has led to the use of streams, rivers and landfills as receptacles of untreated wastes, thus leading to their pollution (Orji et al., 2006). Proper collection and safe disposal of wastewater are recognized as a necessity in an urbanized, Industrialized society (Jawetz and Melux, 1984).

There have been various studies on the antimicrobial properties of plants such as cashew (Awe, 2001) and *Emantia chlorantia* (Gill and Akinwumi, 1986). *Bridelia ferruginea* belongs to the family Euphorbiaceae. It is commonly called Kirni, Kizni (Hausa), Marehi (Fulani), Iralodan (Yoruba), Ola (Igbo), or Kensange abia (Boki). The bark is dark grey, rough and often markedly scaly (Kolawole and Olayemi, 2003). *B. ferruginea* benth is used in African traditional medicine as a decoction of the leaves and is used as a purgative and to treat diabetes (Cimanga et al., 1999).

Although the lungs excrete carbon dioxide, the human kidneys are the major organs responsible for excretion and osmoregulation (Lewis, 1998). This study evaluated the antibacterial activities of *B. ferruginea*, its potential use in the treatment of domestic wastewater and the toxicological effects of the treated wastewater on the kidneys of albino rats.

*Corresponding author. E-mail: tomak74@yahoo.com. Tel: 234(0)8060088495.

MATERIALS AND METHODS

Culture media

The media used for isolation of bacteria from domestic wastewater were Nutrient agar (Oxoid), Eosine Methylene Blue (EMB) and Methyl Red Voges Proskaeur (MRVp) broth. Media were prepared according to the manufacturer's instructions.

Collection of plant material and preparation of plant extracts

B. ferruginea benth bark was collected from the residential quarters of the University of Ilorin, Nigeria. A voucher specimen was deposited at the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria. A methanolic bark extract of *B. ferruginea* was prepared according to the procedure of Kolawole and Olayemi (2003).

Collection of domestic wastewater

Domestic wastewater was collected in sterile 500 ml conical flasks, according to previously described procedures (APHA, 1998), from the female hostel of the University of Ilorin, Nigeria, and quickly transferred to the laboratory for analysis. The pH of the wastewater before and after treatment was determined using a pH meter (Denver Instrument Model 20).

Treatment of domestic wastewater

B. ferruginea bark extract (2.5 g) was dissolved in 100 ml of absolute methanol to yield a concentration of 2.5% (w/v). An aliquot of 10 ml of the stock solution was then used to treat 100 ml of domestic wastewater (10% [v/v]) for varying times of 0, 24, 48, 72 and 96 h. After treatment, the wastewater samples were then filtered through Whatman (No. 1) filter paper in a funnel. The filtrate was then subjected to determination of bacterial counts, determination of pH and toxicological studies using white albino rats. Domestic wastewater treated with absolute methanol alone was designated the positive control, while domestic wastewater not subjected to any treatment was designated the negative control. The positive and negative controls were subjected to the same experimental analysis.

Isolation and enumeration of bacteria before and after wastewater treatment

The total heterotrophic bacteria count (THC) of the wastewater samples before and after treatment was determined on Nutrient agar using the pour plate technique (APHA, 1998). Inoculated plates were incubated at 37°C for 24 h and distinct colonies were counted and recorded as the total bacterial count (cfu/ml). For identification, isolates were subjected to biochemical tests for tentative identification (Buchanans and Gibbons, 1974).

Animal studies

Eighty white male albino rats (*Rattus novergicus*), weighing between 150 - 160 g, were obtained from the animal house of the Faculty of Agriculture, University of Ilorin. Animals were kept in well-ventilated cages in the animal house of the Department of Anatomy, University of Ilorin. The rats were fed (*ad libitum*) for 24 to 48 h to stabilize them before being subjected to the experimental study. The rats were grouped randomly into four groups, each containing twenty albino rats. The animal experimental investigations were performed according to the principles outlined in the Helsinki declaration. After having been fasted overnight, the rats were allocated into treatment groups as follow: Group I: Rats fed with *B. ferruginea* extract-treated wastewater; Group II: Rats fed with untreated waste water (positive control); Group III: Rats fed with absolute methanol-treated wastewater (negative control); and Group IV: Rats fed with only sterile distilled water (standard control). Four rats each in groups I to III were sacrificed 24 h after a daily dose of 5 ml of the group experimented wastewater (Akanji and Nlumanze, 1987), while those in group IV were sacrificed 24 h after a daily dose of 5 ml of sterile distilled water. All groups were fed with double distilled water with Batex feed (commercially formulated feed) after daily doses of administration. Observations were made for a period of 96 h.

Histopathological studies

Histopathological studies on the kidneys of sacrificed rats were carried out according to the method described by Krause (2001).

RESULTS

Analyses of wastewater samples

Twelve bacterial species were isolated from the domestic wastewater before treatment. These were tentatively identified and classified into four families, viz. Enterobacteriacea, Pseudomonadacea, Micrococacea and Bacilliacea. The species comprised *Bacillus subtilis*, *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas frutescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus faecalis*, *Streptococcus lactis* and *Streptococcus pyogens.*

Regarding pH, there was a gradual increase in the pH of the domestic wastewater treated with absolute methanol alone compared to that of the positive control and *B. ferruginea* extract-treated wastewater (Table 1). There was a gradual reduction in the bacterial count as the contact time increased from 0 to 96 h for the *B. ferruginea* extract-treated wastewater. There was also a reduction in the total bacterial count of the *B. ferruginea* extract-treated wastewater compared to the negative and positive controls (Table 2). Notably, the pH and total bacterial count of the standard control (sterile distilled water) was neutral and zero, respectively.

Histopathology

The histopathological findings revealed an acute pyelonephritis with oedematous infiltration of cells in rats fed with *B. ferruginea* extract- treated wastewater from to 96 h. Aggregated polymorphs and disorganized epithelial lining with haemorhage was observed in the tissues of rats designated as positive and negative controls, respectively. However, there was no damage to the tissues of the standard control (Figures 1 - 4).

DISCUSSION

One of the most important steps in wastewater treatment for re-use is coagulation/sedimentation to remove hardness and reduce the bacterial load. In this study, the antibacterial activities of *B. ferruginea* in wastewater treatment and its possible toxicological renal effects on rats fed with the treated water was investigated.

The findings from the study indicated that twelve bacterial species were found to be associated with the domestic wastewater before treatment. The presence of members of the family Pseudomonadacea is suggestive of environmental contamination (Olayemi, 2007). The presence of members of the family Enterobacteriacea in the wastewater may indicate that the water was contami-

Table 1. pH of domestic wastewater before and after treatment at varying contact time.

Contact time (h)	Water samples	Before treatment	After treatment
0	Positive control	7.2	7.1
	Negative control	7.2	9.0
	B. ferruginea extract-treated	7.2	7.3
24	Positive control	7.1	7.2
	Negative control	7.1	9.2
	B. ferruginea extract-treated	7.1	7.2
48	Positive control	7.3	7.2
	Negative control	7.3	9.1
	B. ferruginea extract-treated	7.3	7.1
72	Positive control	7.2	7.1
	Negative control	7.2	9.3
	B. ferruginea extract-treated	7.2	7.1
96	Positive control	7.4	7.3
	Negative control	7.4	9.2
	B. ferruginea extract-treated	7.4	7.0

Table 2. Total bacteria count of wastewater before and after treatment

Contact time(h)	Water samples	Total bacteria count (cfu/ml)	
		Before treatment × 10^3	After treatment × 10^3
0	Positive control	4.85	4.85
	Negative control	4.85	3.75
	B. ferruginea extract-treated	4.85	3.10
24	Positive control	4.80	4.78
	Negative control	4.80	3.90
	B. ferruginea extract-treated	4.80	2.04
48	Positive control	4.72	4.66
	Negative control	4.72	3.48
	B. ferruginea extract-treated	4.72	0.82
72	Positive control	4.60	4.54
	Negative control	4.60	3.24
	B. ferruginea extract-treated	4.60	0.01
96	Positive control	4.48	4.26
	Negative control	4.48	3.10
	B. ferruginea extract-treated	4.48	0.01

nated with faecal matter, while the presence of members of the families Bacillariacea and Micrococacea in the wastewater may be attributed to bath water, which constitutes a major part of the domestic wastewater from the hostels. Their ability to undergo sporulation makes them able to survive adverse conditions (Ogihora, 1988).

Determination of the pH of the wastewater before and after treatment indicated that there was a gradual increase in the pH of the wastewater treated with absolute methanol alone compared to that of the positive control and *B. ferruginea* extract-treated wastewater. The treatment with methanol alone brought a change in the wastewater to an alkaline condition quite different from the neutral condition achieved as a result of treatment with *B. ferruginea* extract which is in conformity with the positive control (Table 2). This could possibly be due to the combinational effects of the tannins (acidic), one of the active constituents in the *B. ferruginea* extract with methanol (which is alkaline in nature). This combinatory effect resulted in an unchanged pH (neutral) observed for the *B. ferruginea* extract-treated wastewater. Kolawole et al*.* (2007) similarly reported that tannins in the methanolic extract of *B. ferruginea* made domestic wastewater to be slightly acidic to neutral pH.

The results from this study revealed that there was a gradual reduction in the total bacterial count as the *B.*

Figure 1. Histological section of a kidney of rat fed with *B. ferruginea* extract-treated wastewater from 0 - 96 h (×200 in haematoxylene and eosin). The plate shows a case of acute pyelonephritis and oedematous infiltration of cells.

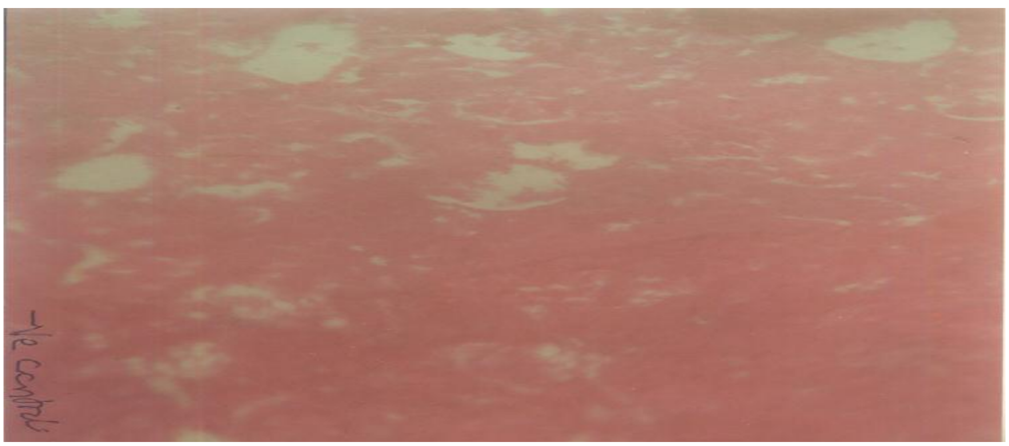

Figure 2. Histological section of the positive control (×200 in haematoxylene and eosin). The plate shows aggregated polymorphs.

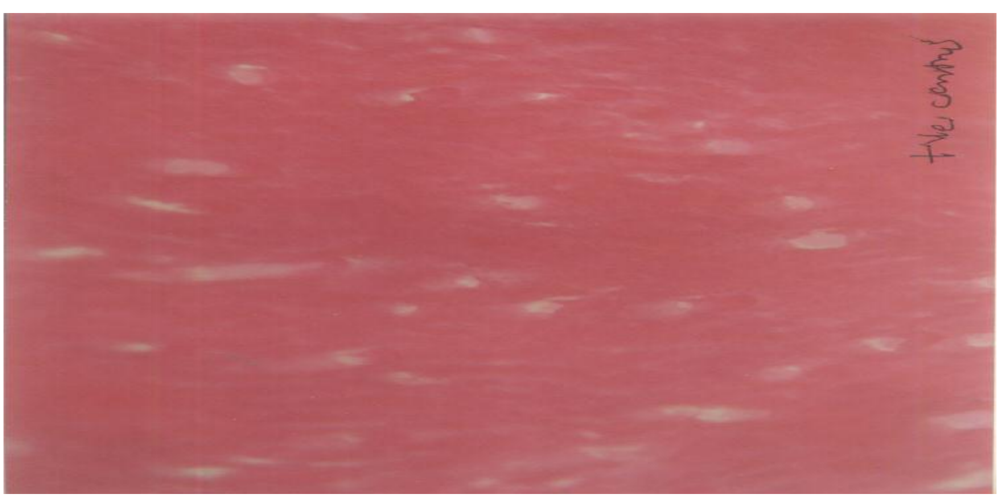

Figure 3. Histological section of the negative control (×200 in haematoxylene and eosin). The plate shows disorganized epithelial lining and haemorrhage.

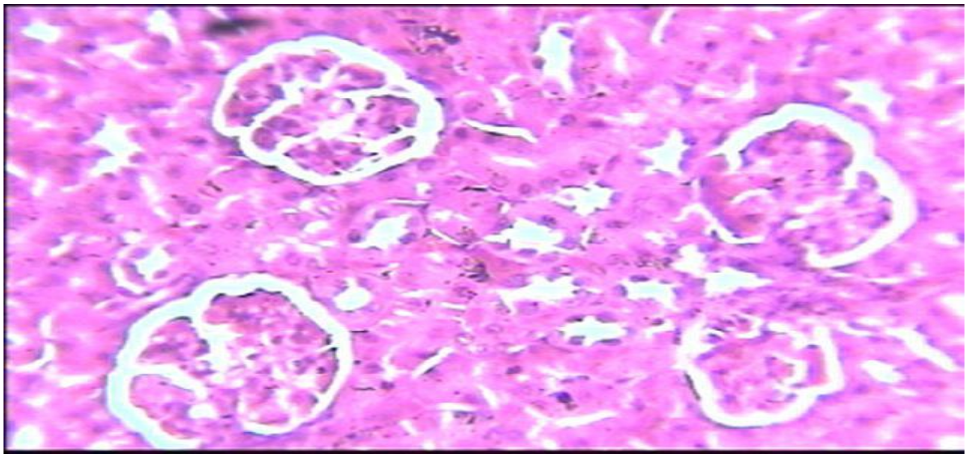

Figure 4. Histological section of the standard control (×200 in haematoxylene and eosin). The plate shows no disorganized tissue and no haemorrhage.

ferruginea extract-treated wastewater contact period increased from 0 to 96 h. This finding is in consonance with the work done by Kolawole et al*.* (2006) who reported an optimum dosage of 2.5% (w/v) of methanol extract of *B. ferruginea* in reducing the coliform load of domestic wastewater. The ability of the bark extract to reduce the total bacterial load in the wastewater can be explained in the light of the ability of the plant extract to achieve a slightly acidic to neutral pH, thereby assisting in the removal of the bacterial load. This finding agrees with the results of Shittu et al*.* (2006) who reported 100% reduction in total bacteria and feacal coliforms of wastewater samples using ground seeds of *Moringa oleiifera.* The mode of action of the plant extract is similar and comparable to the chemical coagulants like aluminiun sulphate and ferric chloride (Aririatu et al*.*, 1999).

The histopathological results revealed an acute pyelonephritis with oedematous infiltration of cells in rats fed with *B. ferruginea* extract-treated wastewater throughout the duration of the experiment. Aggregated polymorphs and disorganized epithelial lining with haemorhage was observed in the tissues of rats designated as positive and negative controls, respectively. However, there was no damage to the tissues of the standard control rats (Figures 1 - 4). Our results suggest that the administration of *B. ferruginea* extract-treated wastewater in rats results in potential damage to the kidneys that may be as a result of unspent tannins in the extract in the water as the contact time increases. This finding is in agreement with the work done by Munuswamy et al. (2008) who reported potential damage to the tissues of kidneys of rats by unspent tannins.

In conclusion, the antibacterial activities of *B. ferruginea* in wastewater treatment have been investigated. The results revealed reductions in the bacterial counts. However, the ingestion of the treated water by rats resulted in kidney damage. Hence, the proposed use of a *B. ferruginea* crude extract for wastewater treatment for drinking purposes may not be advisable.

ACKNOWLEDGMENTS

Special appreciation to the Head and members of staff of the Department of Anatomy, University of Ilorin, for the use of their animal house.

REFERENCES

Akanji MA, Nlumanze SE (1987). Alkaline phosphatase activities following repeated suramnin administration in some rats tissues in relation to their functions. Pharmacol.Toxical 61:182.

APHA (1998). Standard Methods for the Examination of water and wastewater. 20th edition. American Public Health Association, Water Pollution Control Federation, Washington, DC.

Aririatu LE, Gwodia OT, Nwokeocha CC (1999). The bioremediation potential of some local natural coagulants. Nig. J. Microbiol. 13: 65-69.

Awe S (2001). Antimicrobial screening of some medicinal plants used for diarrhea treatment in Ilorin, Nigeria. M. Sc Thesis, University of Ilorin. pp. 20-30.

Bakana P, VanUfford LQ, Beukelman C, Labadie R, Vlietinck AJ (1999). Complement-inhibiting constituents of *Bridelia ferruginea* stem bark. Planta Med. 65: 213-217.

Bashir BA, Adebayo AA (2003). Seasonal variation in water quality and outbreak of water borne diseases in Yola, Nigeria. Int. J. Gender and Health Studies 1(1): 10-15.

Buchanan RE, Gibbon NE (1974). Bergey's Manual of Determinative Bacteriology (8th ed.). Baltimore. Williams and Wilkin Co, London.

Cimanga K, DeBruyne T, Apers S, Pieter L, Totte J, Kambu K, Tona L,

Gill LS , Akinwumi C (1986). Nigeria medicinal plants practice and belief of Ondo people. J. Ethnopharmacol. 18: 257-266.

Hammer IP (1985). Bacteria indicator and health hazards associated with water. Society of Testing and Materials. 287: 311– 400.

Jawetz ED, Melux JL (1984). Bacterial indicator and health hazards associated with water. Society of Testing and Materials. 300: 489-500.

Kolawole OM, Oguntoye SO, Agbede O, Olayemi AB (2006). Studies on the Efficacy of *Bridelia ferruginea* Benth bark extract on reducing the coliform load and B.O.D of Domestic Wastewater. Ethnobotanical leaflets Illinois USA*,* Int. Web J. http://www.siu.edu 10: 228-238.

Kolawole OM, Oguntoye SO, Agbede O, Olayemi AB (2007). Studies on the efficacy of *Bridelia ferruginea* benth bark extract for domestic wastewater treatment. Bulletin of chemical society of Ethiopia. 21(2): 205-211.

Kolawole OM, Olayemi AB (2003). Studies on the Efficacy of *Bridelia ferruginea* benth Bark in water purification. Nig. J. Pure and Appl. Sci. 18: 1387-1394.

Krause WJ (2001). The art of examining and interpreting histologic preparations. A student handbook. Partheton publishing group, UK. pp. 9-10.

Lewis R (1998). Functions of kidney. *In*: life. McGraw-Hill, New York pp. 850.

Munuswamy S, Gnanamani A, Deepa G, Sudha M, Madharacheryulu E, Deivanai K (2008). In vivo studies on evaluation of potential toxicity of unspent tannins using albino rats (*Rattus novergicus*). Food and Chemical Toxicology 46(6); 2288 – 2295.

Ogihora T, Katrina AB, Holgar H (1988). Microbiological Survey of Imeka Shiru pond (Japan). Seasonal fluctuation of sanitary indicator bacteria and analysis of microflora. Bull college of Agriculture and Veterinary Medicine. Nibon University. l 40: 180-189.

Olayemi AB (2007). Crisis of the commons: Global water challenge. Eighty-First Inaugural Lecture, University Of Ilorin. Printed at Unilorin Press pp. 8-12.

Orji MU, Ezenwaje EE, Anyaegbunam BC (2006). Spatial appraisal of shallow well water pollution in Awka, Nigeria. Nig. J. Microbiol. 20(3): 1389-1384. ISSN 0794-1293.

Rowe PS (1994). Factors that influence bacteria in water. J. Appl. Bacteriol. 18: 408-559.

Shittu OB, Akpan I, Okonko IO (2006). Comparative coagulant property of latex of *Calotropis procera* and ground seeds of Moringa Oleifera. Nig. J. Microbiol. 20(3):1419-1426 ISSN 0794-1293.