academicJournals

Vol. 8(26), pp. 915-923, 10 July, 2014
DOI: 10.5897/JMPR2014.5468
Article Number: 7A63EB346407
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Effect of crude root extract from *Synadenium glaucescens* on selected bacterial infections in albino mice *(Mus musculus)*

R. A. Max[1]*, C. Mwageni[2] and G. G. Bakari[1]

[1]Department of Biochemistry and Physiology, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P. O. Box 3017, Morogoro, Tanzania.
[2]Chief Government Chemistry Laboratory, P. O. Box 164, Dar es Salaam, Tanzania.

Received 5 May, 2014; Accepted 4 July, 2014

Studies were carried out to investigate the effect of root extract from a tropical plant *Synadenium glaucescens* against two pathogenic species of bacteria in mice. A total of 120 mice were used in two experiments involving two bacterial species, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In each experiment, 60 mice were randomly divided into 6 groups with or without bacterial infection and with or without root extract. Clinical signs and survival of the mice were monitored; skin, kidneys and livers were examined for gross lesions, histopathological changes and bacterial counts. Results indicated that mice infected with the two bacteria and treated with the root extract from *S. glaucescens* had significantly less ($P < 0.05$) severe skin lesions compared to mice from the untreated groups. Histopathological examination of liver and kidney tissues showed hydropic degeneration and inflammatory reaction in uninfected groups receiving the extracts. Protein in tubular lumens, desquamated tubular epithelium and necrosis around central veins were observed in livers of mice infected with *S. aureus* and treated with the extract at 50 mg/kg body weight. It is concluded that root extract from *S. glaucescens* had significant antibacterial activity against the tested bacteria. Histopathological changes in the kidneys and livers of mice which received the extract alone suggested that high doses of the tested extract could be harmful to the mice. Further studies are needed to find out optimum dosage and whether the extract is harmful to other organs.

Key words: *Synadenium glaucescens*, root extract, antibacterial activity, mice.

INTRODUCTION

Many plants are known to possess medicinal properties and have been used by humans worldwide as natural remedies against various diseases since time immemorial (Ruffo et al., 2002; Amara et al., 2008). Recently, there has been a growing interest in the use of plants and plant products to treat diseases or improve health in humans and animals (Bussmann and Sharon, 2006). According to World Health Organization (WHO, 2007) about 80% of the world population depends on medicinal plants for their health care needs (Gulfraz et al., 2006).

*Corresponding author. E-mail: romso@yahoo.com. Tel: +255 757 104 255. Fax: +255 232 604 647.

Reliable availability, affordability and environmental friendliness are among main reasons for the current increased dependency on plants and their products (WHO, 2007; Yirga, 2010). Moreover, the emergence and spread of pathogens which are resistant to the proprietary antimicrobials calls for a constant search for new drug families. Since plants are among the potential source of novel compounds, scientists and the pharmaceutical industry worldwide have shown deep interest in the field of phytochemistry (Adnan et al., 2010).

Many plants belonging to Euphorbiaceae family have been shown to possess varying medicinal properties and have been documented in the literature (Chika et al., 2007; Kumar and Chaturvedi, 2010, Mwine and Van Damme, 2011). Extracts from the *Euphorbiaceae* plants have been used in wound healing, treatment of ear infections, malaria, diarrhea, heart diseases, diabetes, hemorrhages, hepatitis, jaundice and scabies (Mwine and Van Damme, 2011). In the eastern and southern Africa regions, the plants are used to treat stomach-ache, urinal-genital problems, excessive menstruation, tuberculosis and cardiac palpitations (Melo-Reis et al., 2010). The plant *Synadenium glaucescens*, which belongs to this family, is widely used in Tanzania to treat various diseases including skin conditions, sores and wounds (Chhabra et al., 1984). However, unlike other family members, scientific information about the effectiveness of traditionally prepared *S. glaucescens* extracts against microbial agents is scanty. Studies by Mabiki et al. (2013 a, b) have demonstrated antiviral activity of *S. glaucescens* against several viruses of veterinary importance. The main objective of this study was to investigate the effect of crude root extract of *S. glaucescens* against selected pathogenic bacteria of public health importance using an *in vivo* system.

MATERIALS AND METHODS

Source and collection of the test plant

The test plant (*S. glaucescens*) was sourced from Makambako district [8°51′S 34°50′E] in the southern region of Iringa, Tanzania. About 5 kg of roots were collected from mature trees (ranging from 3 to 5 m in height) into clean paper bags and then transported to our laboratory for processing and preparation for testing. Roots were chosen because they are commonly used by traditional healers. In the laboratory, the roots were washed using sterile water to remove debris and then air-dried on a lab bench for a week before being ground to pass through a 1.5 mm sieve.

Preparation of crude roots extract

Three portions of the air-dried sample, each weighing 30 g were subjected to Soxhlet extraction for 4 h using 99% ethanol. The solvent was then completely removed from the suspension using rotary evaporator. The resultant residue was pulverized to become root extract powder, sealed in a bijou bottle and then stored in a cool dry place until used.

Experimental animals and their management

A total of 120 albino mice, ranging between four and six weeks of age were selected randomly and kept in experimental cages for a week to acclimatize with the experimental conditions. The mice were randomly allocated into groups of 10 animals each and fed on a basal feed (broiler finisher mash) with *ad libitum* access to drinking water. All mice received an oral dose of a broad-spectrum antibiotic (erythromycin) to rid them of any bacterial infections prior to the experiments. On the day of experiment, each mouse in each cage was weighed and number-marked on the back using a permanent marker pen.

Preparation of bacteria broth

Pure cultures of Gram positive bacteria (*Staphylococcus aureus* ATCC25923) and Gram negative (*Pseudomonas aeruginosa* locally isolated strain) were cultivated in nutrient broth media and then serially diluted before an overnight incubation in solid plate count agar. The colonies on the plate agar were counted and the resulting numbers of bacteria were 1.02×10^{9} cfu/ml (*S. aureus*) and 2.60×10^{10} cfu/ml (*P. aeruginosa*). The bacteria broths were kept in universal bottles at 4°C until used (Ayo et al., 2007).

Preparation of injectable root extract

1000 mg of the root extract powder were dissolved in 20 ml of water for injection (that is, 50 mg/ml) before being passed through a 0.2 µm filter membrane to remove larger particulate and bacteria. This injectable preparation was used within 2 h of its preparation.

Grouping and treatment allocation

Two separate experiments were carried out using 60 mice each and one bacterial species at a time (Table 1). Induction of bacterial abscesses was carried out using a method described by Dale et al. (2004) with some modifications that the mice were shaved on the abdomen rather than the dorsal area. Exactly 100 µl of *S. aureus* and *P. aeruginosa* broth were then injected subcutaneously at the shaved abdominal area. The test drug was administered to the mice through intra-peritoneal route (Chika et al., 2007) once the induced abscesses were apparent.

In the first experiment (*S. aureus*), the 60 mice were randomly divided into six groups (n = 10). The first group (G1) served as a negative control, that is, the mice were neither infected with selected bacteria nor receive the test extract. Mice in the second and third groups (G2 and G3) were not infected but received two different doses of the test extract (25 and 50 mg) with no infection. The fourth group (G4) served as a positive control, that is, the mice were infected with *S. aureus* but were not treated with the test extract. The mice in the last two groups (G5 and G6) were also infected with *S. aureus* and then treated with two different doses (25 and 50 mg/kg body weight). The two doses were selected following a pilot experiment using four different doses whereby higher doses showed signs of toxicity to mice.

The second experiment was similar to the first experiment with the only exception that *P. aeruginosa* was used as a test bacterium.

Visual observations

Following the bacterial infections, mice from all groups were monitored twice a day for development of skin abscesses and any other lesions characteristic of each bacterium infection including swelling and redness. After administration of the test extract,

Table 1. Grouping and treatment allocation (mg/kg body weight).

Parameter	Group	Infection	Extract
Experiment 1 (*S. aureus*)	G1	No	0
	G2	No	25
	G3	No	50
	G4	Yes	0
	G5	Yes	25
	G6	Yes	50
Experiment 2 (*P. aeruginosa)*	G1	No	0
	G2	No	25
	G3	No	50
	G4	Yes	0
	G5	Yes	25
	G6	Yes	50

observations on clearance of the abscesses were monitored to the end of the experiments. Five mice from each group were sacrificed and organs (skin, kidney and liver) were taken and preserved for bacterial count and histological examinations.

Assessment of mice survival

Deaths of mice were recorded for up to 7 days after commencement of the treatments as an index of survival. Mean survival rate of mice that died was calculated by dividing the number of remaining live mice by the original total number of mice in the group.

Bacterial counts in tissues

Assessment of the number of bacteria present in the sampled tissues was done using a standard technique described by Miles et al. (1938) using nutrient agar and Baird Parker media for *P. aeruginosa* and *S. aureus*, respectively. Mice were sacrificed and the abdominal cavities were opened (aseptically) to remove kidney and liver; each organ was weighed and then 1 g was homogenized in 2 ml sterile normal saline. Skin lesions were sampled by first disinfecting the skin with 70% ethanol followed by removal of the infection site and underlying tissue using a sterile scalpel blade. One gram of the removed skin lesions was homogenized in 2 ml sterile normal saline. The homogenates (1 ml) of each tissue were serially diluted 10 folds in normal saline before pouring on plates and incubating at 37°C for 24 h. Bacterial counts were expressed as numbers of *S. aureus* or *P. aeruginosa* colony forming units per gram per ml (cfu $g^{-1}ml^{-1}$) of tissue. A total of 144 samples were examined.

Histopathological examination of tissue samples

The remaining portions of kidney and liver were processed for histopathological examination using a standard procedure. Initially, tissues were grossly examined and then five micrometer thick sections were fixed in 10% buffered formalin, embedded in paraffin, stained with hemotoxylin-eosin (HE) and examined under light microscope. Tissue samples were evaluated for degree of deformation, infiltration of cells, protein formation in tubules, and necrosis. The histopathological pictures of tissues from the different animal groups were assessed blindly by a pathologist. Pictures of the kidney and liver cells were taken using a still digital camera.

Statistical analysis

All data obtained from the study were subjected to one way analyses of variance (ANOVA). A value of $P < 0.05$ was considered significant using the MS Excel (2007) package.

RESULTS

Bacteria-induced lesions

Following the inoculation of the bacteria in mice, appearance of small skin lesions and abscesses occurred within 24 to 48 h of infection in G4 (infected + not treated) of both experiments (Figures 1 and 2). Infection due to *P. aeruginosa* was different from that of *S. aureus* in that the former had burnt appearance skin lesions whereas visible skin and kidney abscesses (Figure 3) were the features seen in *S. aureus* infected mice. Clinical signs such as rough coat, hunched back, ocular discharge, moribund state and some deaths were observed. Close visual observation of the skin lesions of mice from G5 and G6 (infected + treated) showed disappearance of pus discharges from the skin within seven days of infection. In both experiments, all mice treated with the extract showed varying degrees of improvement on the skin lesions as compared to those mice in G4.

Mice survival assay

Results of the mice survival assay are shown in Table 2. In the first experiment all mice infected with *S. aureus* in a group which was not infected and not treated (G1) and in group which was infected and not treated (G4) the mice survived to the end of observation period. Two mice

Table 2. Effect of different doses of *S. glaucescens* root extract on the survival of mice infected with *S. aureus* or *P. aeruginosa.*

Parameter	Group	Infection	Extract	Death
	G1	No	0	0
	G2	No	25	2
Experiment 1	G3	No	50	2
(*S. aureus*)	G4	Yes	0	0
	G5	Yes	25	3
	G6	Yes	50	4*
	G1	No	0	0
	G2	No	25	2
Experiment 2	G3	No	50	2
(*P. aeruginosa*)	G4	Yes	0	1
	G5	Yes	25	5*
	G6	Yes	50	7*

Table 3. Bacteria counts from different mice organs following induced infections and treatment with different doses of *S. glaucescens* crude root extract.

Parameter	Group	Infection	Extract	Mean (Cfu/ml)		
				Skin	Kidney	Liver
	G1	No	0	3.0	2.6	4.0
	G2	No	25	4.8	0.0	0.0
Experiment 1	G3	No	50	2.4	0.0	0.0
(*S. aureus*)	G4	Yes	0	59.0	92.0	37.0
	G5	Yes	25	21.0	6.0	5.2
	G6	Yes	50	22.8	2.0	8.6
	G1	No	0	2.2	2.4	2.2
	G2	No	25	4.8	0.0	0.0
Experiment 2	G3	No	50	2.4	0.0	0.0
(*P. aeruginosa*)	G4	Yes	0	104.0	28.2	79.2
	G5	Yes	25	2.2	0.4	0.0
	G6	Yes	50	27.7	2.3	3.7

in each group (G2 and G3) (not infected and treated) died and the remaining 8 mice in each of these groups survived to the end of observation period. Seven mice in G5 (infected and treated) survived and 3 died before the end of observation period while only 6 mice survived in G6 (infected and treated).

In the second experiment (Table 2) all mice in G1 (not infected and not treated) survived, 2 mice in each G2 and G3 (not infected and treated) died. One mouse, five and seven mice died in G4 (infected and not treated), G5 (Infected and treated) and G6 (infected and treated), respectively.

Bacterial counts

Bacterial counts (cfu/ml) in different organs of mice following treatment with two different levels of test extract are presented in Table 3. In both experiments, cfu/ml of organs from mice in G1 to G3 (no infection) were far less regardless of extract administration. Conversely, bacterial counts of G4 (infection without treatment) groups were significantly higher than those treated with either 25 or 50 mg extract per kg body weight. Regression between the dose of extract and bacterial counts was found to be negative (R^2 = 0.68; P = 0.37), that is, the counts decreased with increasing dose of the extract.

Histopathological findings

These results are summarized in Tables 4 and 5. Kidneys and livers from mice in the control groups (G1, not infected and not treated) were normal. In G2 (no infection,

Table 4. Histopathological findings of livers and kidneys of chickens infected with *S. aureus* and treated with *S. glaucescens* root extract.

Infection	Group	Extract	Kidney		Liver	
			Changes	**Severity**	**Changes**	**Severity**
No	G1	0	Desquamation of tubular epithelium	NO	Vacuolar or hydropic degeneration	NO
	G2	25		+		+
	G3	50		+		+
	G1	0	Protein deposition in tubular lumen	NO	Necrosis	NO
	G2	25		+		NO
	G3	50		+		NO
	G1	0	Inflammatory reaction (mono-nuclear cells)	NO	Inflammatory reaction (mono-nuclear cells)	NO
	G2	25		+		+
	G3	50		+		+
	G1	0	Glomerular tufts	NO	-	
	G2	25		+		
	G3	50		+		
Yes	G4	0	Desquamation of tubular epithelium	NO	Vacuolar or hydropic degeneration	NO
	G5	25		+		+
	G6	50		++		+++*
	G4	0	Protein deposition in tubular lumen	NO	Necrosis	NO
	G5	25		+		NO
	G6	50		+++		NO
	G4	0	Inflammatory reaction (mono-nuclear cells)	NO	Inflammatory reaction (mono-nuclear cells)	NO
	G5	25		NO		+
	G6	50		NO		++
	G4	0	Glomerular tufts	NO	-	
	G5	25		NO		
	G6	50		NO		

NO: Not observed.

infection, 25 mg extract) and G3 (no infection, 50 mg extract) of both experiments, their liver cells had hydropic degeneration and inflammatory reactions. In the kidneys, there were protein depositions in tubular lumen (Figure 4), glomerular tufts (Figure 5) and desquamation of the tubular epithelium.

Protein deposition in the tubular lumen and inflammatory reaction in kidney (Figure 4) were observed in mice which were infected with *S. aureus* then treated with the extract at a dose of 50 mg/kg body weight. The livers showed the presence of inflammatory reaction and vacuolar or hydropic degeneration (Figure 6). Tissue sections from mice that received 25 mg of the extract (G2 and G5), showed mild desquamated tubular epithelium and protein in the tubular lumen of kidneys. Liver tissue showed mild hydropic degeneration and inflammatory reaction. Groups infected with *P. aeruginosa* then treated at dose of 50 mg (G6) had livers with hydropic degeneration, necrosis formation (Figures 6 and 7) and mononuclear cells inflammation. However, kidneys were normal. For those infected and treated with 25 mg extract (G5), appearance of early stages of vacuolation and mononuclear cells infiltration in liver were observed while their kidneys were normal.

DISCUSSION

The aim of the current study was to investigate the effect of crude ethanolic extract from roots of *S. glaucescens*

Table 5. Histopathological findings of livers and kidneys of chickens infected with *P. aeruginosa* and treated with *S. glaucescens* root extract.

Infection	Group	Extract	Kidney		Liver	
			Changes	Severity	Changes	Severity
No	G1	0	Desquamation of tubular epithelium	NO	Vacuolar or hydropic degeneration	NO
	G2	25		+		+
	G3	50		+		+
	G1	0	Protein deposition in tubular lumen	NO	Necrosis around central vein	NO
	G2	25		+		NO
	G3	50		+		+++*
	G1	0	Inflammatory reaction (mono-nuclear cells)	NO	Inflammatory Reaction (mononuclear cells)	NO
	G2	25		+		+
	G3	50		+		+
	G1	0	Glomerular tufts	NO	-	
	G2	25		+		
	G3	50		+		
Yes	G4	0	Desquamation of tubular epithelium	NO	Vacuolar or hydropic degeneration	NO
	G5	25		NO		NO
	G6	50		NO		+++*
	G4	0	Protein deposition in tubular lumen	NO	Necrosis around central vein	NO
	G5	25		NO		NO
	G6	50		NO		+++*
	G4	0	Inflammatory reaction (mono-nuclear cells)	NO	Inflammatory Reaction (mononuclear cells)	NO
	G5	25		NO		+
	G6	50		NO		+++*
	G4	0	Glomerular tufts	NO	-	
	G5	25		NO		
	G6	50		NO		

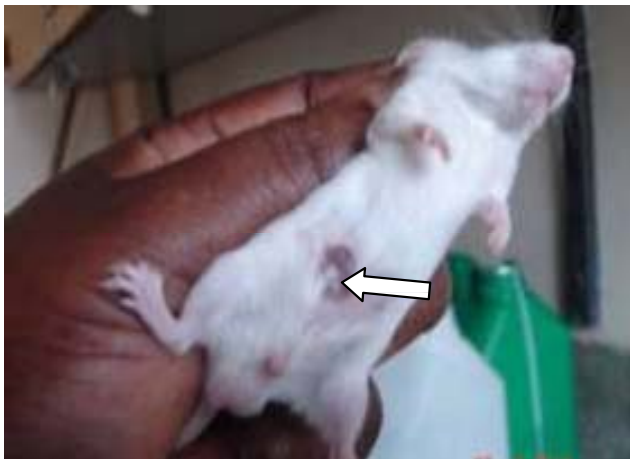

Figure 1. Skin lesion caused by *P. aeruginosa* (the arrow shows a burnt appearance skin lesion).

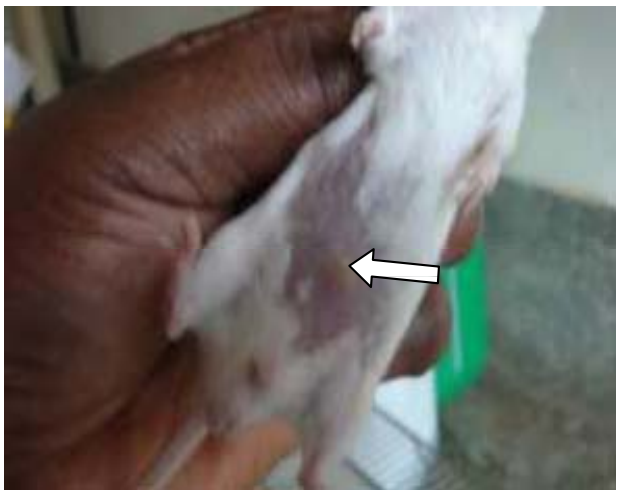

Figure 2. Skin abscesses caused by *S. aureus* infection.

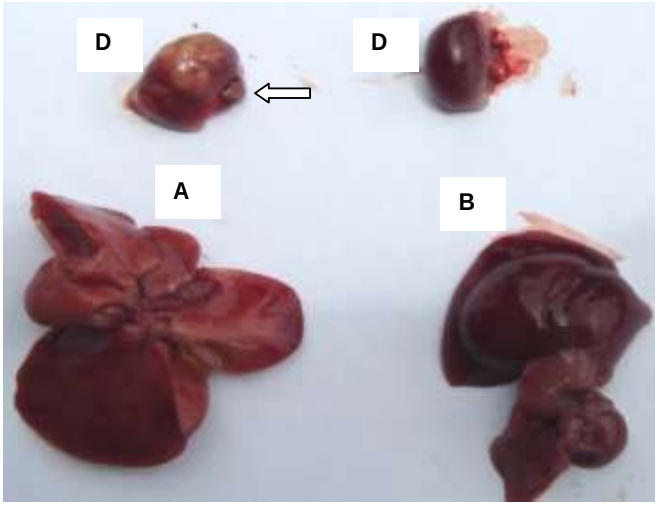

Figure 3. Abscesses in kidney due to *S. aureus* infections (A); normal kidney (B) liver infected by *P. aeruginosa* (C) and normal liver (D).

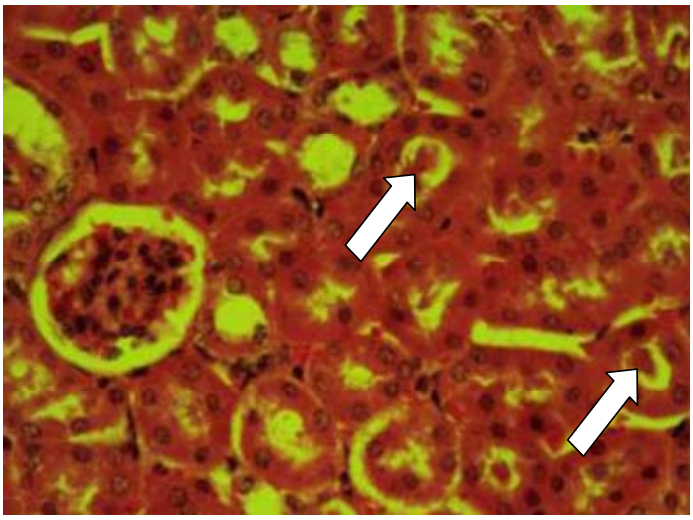

Figure 4. A representative section showing tubular lumen protein in kidneys from bacteria- infected-groups which received 50 mg root extract/kg body weight.

against selected pathogenic bacteria using an *in vivo* assay. Results indicated that induction of bacterial infections to mice lead to development of clinical signs and lesions characteristic of the test bacteria. On treatment with two different doses of the crude extract, it was clearly observed that the severity of clinical signs and lesions were significantly reduced. For instance, mice in groups receiving the extract showed disappearance of pus discharges from the abscesses as compared to those in the positive control groups. This was a clear indication that the extract had a negative effect on the development of bacterial infections. These observations

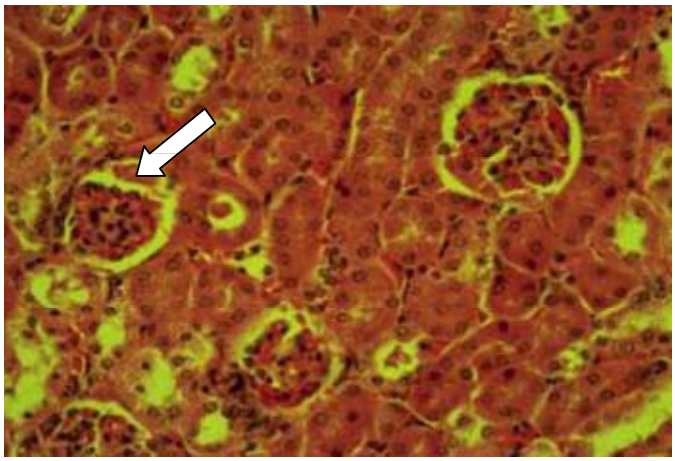

Figure 5. A representative section showing protein in glomerular tuft from bacteria-infected- groups which received 50 mg root extract per kg body weight.

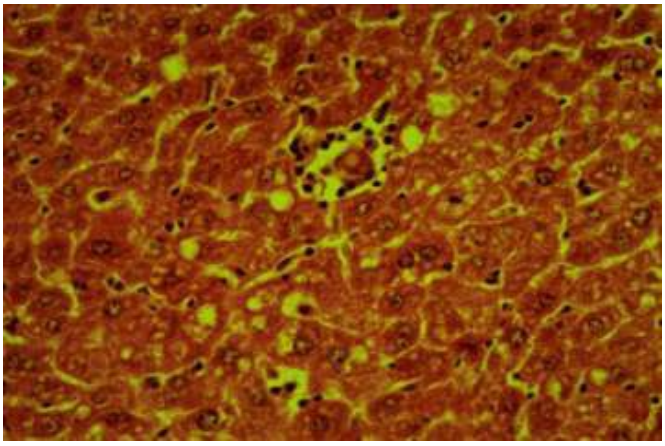

Figure 6. Hydropic degeneration of the hepatocytes from the *P. aeruginosa* infected group which received 50 mg extract/kg body weight.

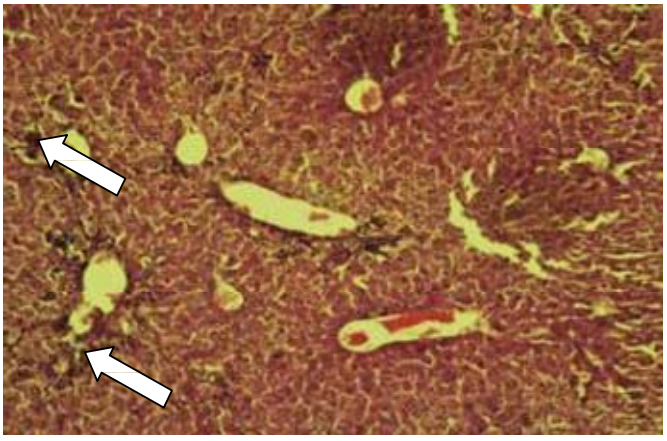

Figure 7. Liver necrosis around central vein from the *P. aeruginosa*-infected-group which received 50 mg extract/kg body weight

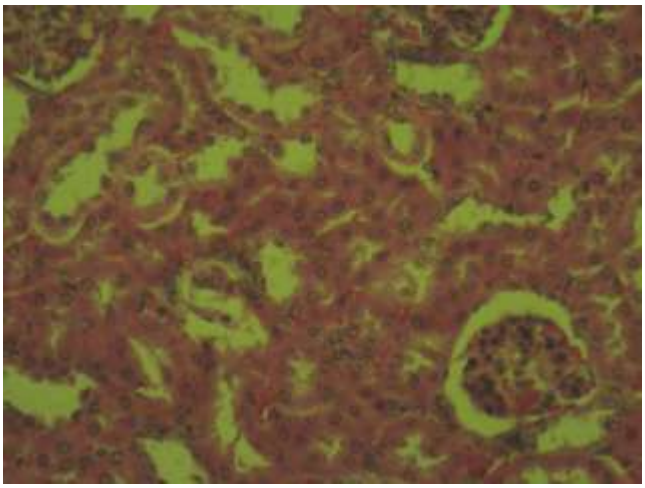

Figure 8. Histological section of a normal mouse kidney.

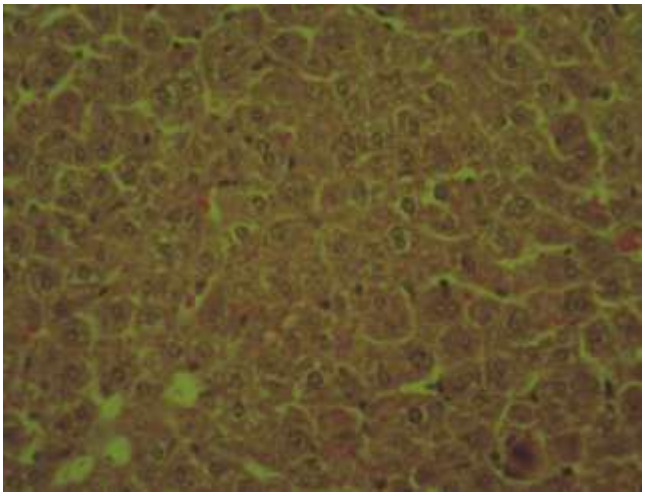

Figure 9. Histological section of a normal mouse liver.

were consistent with those from other studies on plants with antibacterial activities (Valgas et al., 2007; Abu-Al-Basal, 2009). Further antibacterial activity was demonstrated from the results of tissue bacterial counts and histopathological examinations of kidney and livers. In both experiments, mice infected with bacteria without treatment with the extract had significantly higher tissue bacterial counts than their treated counterparts (Table 3). This was an indication that the extract had a direct antibacterial effect on the test bacteria in their hosts. The presence of higher bacterial counts in skin than in the internal organs (kidneys and lives) can be explained by the fact that skin harbors large number of bacteria as normal flora (Kumari et al., 2009).

Histopathological examination of kidney and liver tissues indicated changes attributed to both extract and test bacteria (Figures 8 and 9). Administration of the extract induced a degree of desquamation of tubular epithelium, protein deposition, inflammatory reaction and formation of glomerular tufts in the kidney. In the liver, necrosis around central veins and vacuolar degeneration were features associated with the extract administration. These findings suggest that the *S. glaucescens* extract could have some pathological effects on the mice's internal organs (Melo-Reis et al., 2010).

Two treatment doses were used to determine the optimum dose and whether the extract effect was dose-dependent or not. The current findings indicated a weak dose dependent effect as deduced from the regressions coefficient (R^2 = 0.68, P = 0.37, that is, the antibacterial activity increased with increasing dose of the extract. Since more mice deaths occurred when the dose was increased from 25 to 50 mg/kg bodyweight, especially with *P. aeruginosa* infection, it is suggested that high doses of the extract could be lethal.

In the current study, it was also observed that more mice deaths occurred when the extract was administered to *P. aeruginosa* infected groups than to those infected with *S. aureus* suggesting that the former bacterium became more virulent on administering the extract. Explanation behind this observation is not clear. However, it is known that *P. aeruginosa* tend to produce toxic compounds as a defense mechanism when confronted by stressful agents like drugs (Adonizio et al., 2008). These compounds, which include LasA protease, LasB elastase, pyoverdin, pyocyanin, alginate as well as toxins, are known to worsen the host animal clinically and may eventually lead to death.

It is concluded that the crude root extract from *S. glaucescens* had significant antibacterial activity against the test bacteria and that the higher dose of 50 mg/kg body weight was associated with some pathological changes in the kidney and liver. The data obtained in this study seem to justify the traditional use of the plant in treating various bacterial infections. Further studies are required, to find out the most optimal dose of root extract in treating infections caused by the tested bacteria in different animal species.

ACKNOWLEDGEMENTS

This study has been funded by the Belgium Technical Cooperation (BTC). The authors wish to thank the people who assisted at various stages of the work including laboratory technicians at the Faculty of Veterinary Medicine, Sokoine University of Agriculture.

Conflict of interest

The authors declare that they have no conflict of interest with any findings in this study. Experiments were carried out in accordance to ethical guidelines of the Sokoine University of Agriculture.

REFERENCES

Abu-Al-Basal MA (2009). *In vitro* and *in vivo* anti-microbial effects of *Nigella sativa*. Seed extracts against clinical isolates from skin wound infections. Am. J. Appl. Sci. 6:1440-1447.

Adnan M, Hussain J., Tahir Shah, MT, Shinwari ZK, Ullah F, Bahader A, Naeem Khan N, Khan AL, Watanabe T (2010). Proximate and nutrient composition of medicinal plants of humid and sub-humid regions in north-west Pakistan. Res. J. Med. Plant 4:339-345.

Adonizio A, Kok-Fai K, Mathee K (2008). Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* by South Florida plant extracts. Antimicrob. Agents Chemother. 52:198–203.

Ayo RG, Amupita, JO, Zhao Y (2007). Cytotoxicity and antimicrobial studies of 1, 6, 8- trihydroxy-3-methyl-anthraquinone (emodin) isolated from the leaves of *Cassia nigricans* Vahl. Afr. J. Biotechnol. 6:1276-1279.

Bussmann RW, Sharon D (2006). Traditional medicinal plant use in northern Peru tracking two thousand years of healing culture. J. Ethnobiol. Ethnomed. 2:47-50.

Chhabra SC, Uiso FC, Mshiu EN (1984). Phytochemical screening of Tanzanian medicinal plants. J. Ethnopharmacol. 11:157-179.

Chika CO, Jude NO, Ifeanyi CO, Beatrice NA (2007). Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*. J. Am. Sci. 3:11-16.

Dale RMK, Schnell G, Wong JP (2004). Therapeutic efficacy of antibiotics against burn wound infection by *Pseudomonas aeruginosa.* Antimicrob. Agents Chemother. 48:2918-2923.

Gulfraz M, Waheed A, Mehmood S, Ihtisham M (2006**)**. Extraction and purification of various organic compounds in selected medicinal plants of Kotli Sattian District, Rawalpindi, Pakistan. Ethnobot. Leafl. 10:13-23.

Kumar GP, Chaturvedi A (2010). Ethnobotanical Observations of Euphorbiaceae species from Vidarbha Region, Maharashtra, India. Ethnobot. Leafl.14:674-680.

Kumari S, Harjai K, Chhibber S (2009). Bacteriophage Treatment of Burn Wound Infection Caused by *Pseudomonas aeruginosa* PAO in BALB/c mice. Am. J. Biomed. Sci. 1:385-394.

Mabiki FP, Mdegela RH, Mosha RD, Magadula JJ (2013b). Antiviral activity of crude extracts of *S. glaucescens* (Pax) against infectious bursal disease and fowl pox virus. J. Med. Plants Res. 7:871-876.

Mabiki FP, Mdegela RH, Mosha RD, Magadula JJ (2013a). *In ovo* antiviral activity of *S. glaucescens* (pax) crude extracts on Newcastle disease virus. J. Med. Plants Res. 7:863-870.

Melo-Reis PR, Andrade LS, Silva CB, Araújo LMM, Pereira MS, Mrue F, Chen-Chen L (2010). Angiogenic activity of *Synadenium umbellatum* pax latex. Brazil. J. Biol. 70:189-194.

Miles AA, Misra SS, Irwijn O (1938). The estimation of the bactericidal power of the blood. J. Hyg. 38:732-736.

Mwine J, Van Damme P (2011). *Euphorbia tirucalli* L. (Euphorbiaceae) The miracle tree: Current status of available knowledge scientific research and essays. Acad. J. 6:4905-4914.

Ruffo CK, Birnie A, Tengnäs B (2002). Edible wild plants of Tanzania. Regional Land Management Unit (RELMA). Technical Handbook Series 27. English Press, Nairobi, Kenya. pp. 766-771.

Valgas C, Machado de Souza S, Smânia EFA, Smânia A Jr. (2007). Screening methods to determine antibacterial activity of natural products. Brazil. J. Microbiol. 38:369-380.

WHO (2007). Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. WHO Library Cataloguing-in-Publication Data pp. 1-5.

Yirga G (2010). Use of traditional medicinal plants by indigenous people in Mekele town, capital city of Tigray regional state of Ethiopia. J. Med. Plants Res. 4:1799-1804.