Research Article

Effects of Methanol Extract of *Morinda lucida* Leaves in *Staphylococcus aureus* Induced Infection in Albino Rats

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Received: 08-05-2018; **Revised:** 02-03-2019; **Accepted:** 18-04-2019.

ABSTRACT

Antibiotic resistance occurs when bacteria change its response to the use of a particular drug and is one of the biggest threats to global health. It occurs naturally but misuse of antibiotics in humans and animals exacerbates the process. The *in-vivo* and *in-vitro* efficacy of methanol extract of *Morinda lucida* leaves against methicillin resistant *Staphylococcus aureus* (MRSA) were investigated in albino rats. Albino rats (n = 25) were grouped into 5 groups (groups A-E). Groups A-D was intraperitoneally induced with $10^6\mu$l of MRSA for 7 days. After which groups A-C orally received graded-doses of *Morinda lucida* leave extract (A = 500, B = 1000 and C = 2000) mg/kg bodyweight while group D received gentamycin antibiotics for 14 days. Group E served as the control. Blood samples were collected for haematological indices and spleen harvested for histological studies. Phytochemical and antibacterial susceptibility of the extract were also investigated. Phytochemical analysis revealed the presence of athroquines, alkaloids, saponins, cardiac glycoside, tannins and sterol in varying amounts. Haemoglobin (HB), white blood cell (WBC) and platelets increased significantly ($p < 0.05$) in groups A-C while in group D, haemoglobin and white blood cell significantly decreased and increased ($p < 0.05$) respectively compared to control. Group A revealed intact central arteries, moderately increased white pulps and moderate inflammatory infiltration in red pulp areas. Groups B-C revealed fairly intact tissue histomorphology. Group C revealed mildly increased white pulps and mild inflammatory infiltrations in red pulp. Group D showed evidence of tissue disruption. The antibacterial susceptibility of the extract exhibited the highest inhibitory effect on *Staphylococcus aureus* at the concentrations of 12.5 mg/ml. Methanol extract of *Morinda lucida* leaves demonstrated efficacy against methicilin resistant *Staphylococcus aureus*.

Keywords: *Morinda lucida*, *Staphylococcus aureus*, methicillin, antibacterial, antibiotic resistance.

INTRODUCTION

Medicinal plants contain secondary metabolites which are used for therapeutic purposes and have been applied for several decades to serve as precursors for the synthesis of new drugs [1]. At present, it is estimated that about 80% of the world population rely on plant preparations as medicines to meet their health needs [2]. Herbs and spices are generally considered safe and effective for the treatment of ailments due to presence of chemical bioactive compounds such as flavonoids, alkaloids, tannins and phenolic compounds [3].

Methicillin-resistant *staphylococcus aureus* was first detected in Britain in 1961 [4]. Some bacteria are naturally resistant to certain types of antibiotics while some bacteria may develop resistance either by genetic mutation or by acquiring resistance from another bacterium. Different genetic mutations yield different types of resistance. For example, some mutations enable the bacteria to produce potent enzymes that inactivate antibiotics, while other mutations destroy the cell target that the antibiotic attacks [5]. Others block the entry ports that allow antibiotics into the cell [6-7] and others manufacture pumping mechanisms that makes the antibiotic to never reach its target [8-10]. Bacteria can acquire antibiotic resistance genes from other bacteria in several ways which includes undergoing a simple mating process called conjugation which involves the transfer of genetic material, including genes encoding resistance to antibiotics from one bacterium to another. Viruses are another mechanism for passing resistance traits between bacteria. The resistance traits from one bacterium are packaged into the head portion of the virus and the virus then injects the resistance traits into any new bacteria it attacks.

It is well documented that *M. lucida* leaves extract has various therapeutic benefits with no known adverse effect among the users. However, the responses of various organs especially liver and kidney of humans to ingestion of this extract remain largely unknown. In search of natural/alternative remedy to the problem of antibiotic resistance, we decided to investigate the activity of methanol extract of *M. lucida* leaves against *staphylococcus aureus* induced infection in albino rats. The aim of the study was to investigate the *in-vivo* and *in-vitro* efficacy of methanol extract of *M. lucida* leaves against MRSA in albino rats. The specific objectives were to determine the phytochemical constituents,

haematological and histological effects on rats and antibacterial susceptibility of the *M. lucida* extract.

MATERIALS AND METHOD

Collection and processing of *Morinda lucida* Leaves

The leaves of *M. lucida* were collected from Nkanu in Enugu State. The leaves of were authenticated by a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. Voucher sample of the leaves were kept at the herbarium of University of Nigeria Nsukka for future references. The leaves were shade-dried at room temperature and pulverized into coarse powder using grinding machine.

Methanol extraction

Five hundred grams of the blended *M. lucida* leaves were soaked in 2000 ml of methanol for 24 hours. The mixture was shook at two hourly intervals and then sieved with muslin cloth and finally filtered with Whatman No. 1 filter paper. The filtrate was concentrated by evaporation and preserved at 4^0C.

Phytochemical Analysis

The dried ground leaves were subjected to preliminary phytochemical analysis for athroquines, alkaloids, saponins, tannins, cardiac glycosides, sterol, flavonoids and resins using standard method as described by Ioan [11].

Collection of Bacterial isolates

The test bacterium, *Staphylococcus aureus* was obtained from the Medical Microbiology Department of University of Nigeria Teaching Hospital Ituku-Ozalla Enugu. The isolate was obtained from the urine of a patient with urinary tract infection. It was further characterized using cultural characteristics, catalase test and coagulase test. The *Staphylococcus aureus* isolate was further confirmed as MRSA by its resistance to oxacillin, ofloxacin, cloxacillin.

In-vitro antibacterial activity

The in-vitro antibacterial activity of the *Staphylococcus aureus* was determined against the methanol extract. Agar well diffusion technique was employed using Muller hinton agar. Briefly, the methanol extract of *M. lucida* was serially diluted from 100 mg/ml to 0.2 mg/ml. The agar was flooded with *Staphylococcus aureus* culture grown on peptone water and allowed to permeate onto the agar for about 2 minutes, the excess was poured out. Using a stand cork borer, wells were created on the Mueller Hington agar plate equidistant from each other. Then the base holes were layered with molten Mueller Hinton Agar so that extract dilutions does not leak. Then the wells were filled with the respective dilutions of the extract and labelled. It was then incubated at 37^0C for 24 hrs. The extract dilutions that showed clear zones of inhibition were regarded as sensitive to the bacterium.

In-vivo antibacterial activity

Albino rats (n = 25), aged 6-months, were purchased from Department of Physiology, University of Nigeria Enugu Campus and were allowed to acclimatize at the animal house of University of Nigeria Teaching Hospital, Enugu for one week. They were divided into 5 groups (groups A-E) of 5 rats each, in accordance with their closest weight.

The methicillin resistant *Staphylococcus aureus* (MRSA) isolate was grown on peptone water and the bacterial load was adjusted to 1 x 10^6 after counting with improved neubar counting chamber. The rats in groups A-D were induced intra-peritonealy with 1 x 10^6 of MRSA while those of group E served as normal control. After 7 days of infection, the rats were then treated with the methanol extract of *M. lucida* and gentamycin antibiotics as follows: groups A-C orally received graded-doses of the extract (A = 500, B = 1000 and C = 2000) mg/kg bodyweight while group D received gentamycin antibiotics for 14 days. Blood samples (3.0 ml) were collected from the rats via the orbital plexus into tri-potassium ethylene diamine tetra acetic acid solution for haematological analysis. The rats were anaesthetized and spleen were harvested and fixed in 10% formal saline for histological analysis.

Haematological analysis

Haematological parameters (haemoglobin, white blood cell, neutrophil, lymphocyte, eosinophil, monocyte, basophil and platelet) were analysed for using haematological auto analyser (Sysmex KX-21N) following manufacturers guideline.

Histological processing

Spleen tissues fixed in 10% formal saline were processed histologically and sections stained by haematoxylin and eosin. The stained sections were examined and photomicrographs obtained.

Statistical analysis

Data were subjected to descriptive statistics and analysed using Student's t-test and analysis of variance at 95% confidence interval. Probability level less than 0.05 was considered statistically significant.

RESULTS

Phytochemical analysis of the extract revealed the presence of athroquines, alkaloids, saponins, cardiac glycoside, tannins and sterol (Table 1).

For the haematological parameters, haemoglobin, white blood cell and platelets increased significantly ($p < 0.05$) in groups A-C when compared with the control (group E). In group D, haemoglobin decreased significantly ($p < 0.05$) while the white blood cell increased significantly ($p < 0.05$) when compared with the control (table 2).

For *In-vitro* sensitivity, the antibacterial susceptibility of methanol extract of *M. lucida* exhibited an inhibitory effect on the *Staphylococcus aureus*. At the concentrations of 12.5mg/ml, the extract showed highest

inhibitory effect. The gentamycin antibiotics also showed significant inhibitory effect.

Histological studies of spleen from the various treatment groups were as shown in figures 1-5. Group A revealed intact central arteries, moderately increased white pulps and moderate inflammatory infiltration in red pulp areas (figure 1). Groups B revealed fairly intact tissue histomorphology (figure 2). There were mildly increased white pulps and mild inflammatory infiltrations in red pulp of group C (figure 3). Group D showed evidence of tissue disruption (figure 4). The control group E showed normal histoarchitecture of the spleen (figure 5) with normal central artery, normal lymphatic aggregations around the central arteries known as white pulp (WP) and normal red Pulp (RP).

Table 1: Phytochemical Analysis *Murinda lucida*

Plant substances	Degree of presence
Athroquines	+++
Alkaloids	+++
Saponins	+++
Tannins	+
Cardiac glycosides	++
Sterol	+
Flavonoids	-
Resins	-

Keys:- Absent, + trace, ++, Moderate, +++Abundant

Table 2: Haematological parameters of rats administered with graded doses of *Morinda lucida* leaves extract.

Haematological parameters	Group A	Group B	Group C	Group D	Group E
HB (g/dl)	14.0 ± 0.7*	14.3 ± 0.2*	14.7 ± 0.8*	9.3 ± 0.2*	12.6 ± 0.3
WBC ($x10^9$/l)	10.8 ± 0.6*	10.2 ± 0.3*	8.1 ± 0.5*	15.0 ± 0.8*	6.8 ± 0.2
Neutrophil (%)	62 ± 3	58 ± 1	52 ± 1	65 ± 2	50 ± 1
Lymphocyte (%)	27 ± 2	36 ± 2	44 ± 1	28 ± 1	46 ± 1
Eosinophil (%)	3 ± 1	2 ± 0.5	2 ± 0.2	3 ± 1	2 ± 1
Monocyte (%)	8 ± 2	4 ± 1	2 ± 1	4 ± 2	2 ± 0.4
Basophil (%)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Platelet ($x10^9$/l)	132 ± 20*	128 ± 9*	125 ± 10*	115 ± 17	110 ± 15

Key: * ($p < 0.05$) Significant

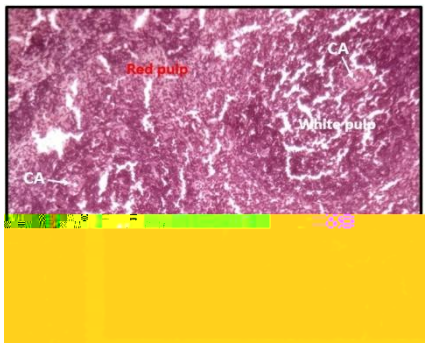

Figure 1: Spleen section photomicrograph of rat infected with *S. aureus* and treated with 500 mg/kg body weight of *Morinda lucida* leaves extract (group A).

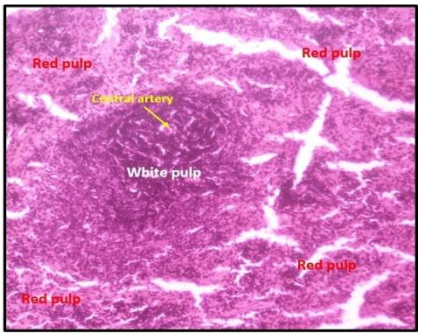

Figure 3: Spleen section photomicrograph of rat infected with *S. aureus* and treated with 2000 mg/kg body weight of *Morinda lucida* leaves extract (group C).

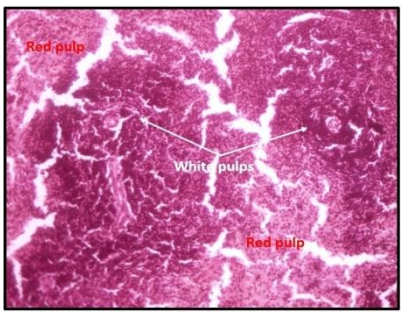

Figure 2: Spleen section photomicrograph of rat infected with *S. aureus* and treated with 1000 mg/kg body weight of *Morinda lucida* leaves extract (group B).

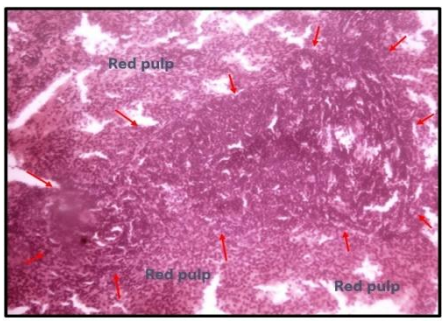

Figure 4: Spleen section photomicrograph of rat infected with *S. aureus* and treated with gentamycin antibiotics (group D).

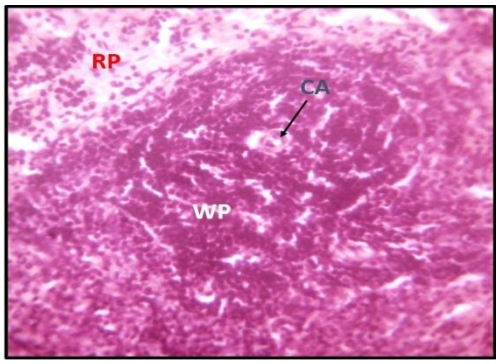

Figure 5: Spleen section photomicrograph of control rat showing normal histoarchitecture of the splenic tissue (group E).

DISCUSSION

Medicinal plants play a key role in health care system worldwide. It has been estimated that approximately 80% of the population in developing countries rely on traditional medicine because of its availability and at little or no cost[12]. Stem bark, leaves and roots of *Morinda lucida* are widely used in folk medicine in treatment of illnesses. The assessment of the extract *in-vitro* showed that the extract was effective against *MRSA* at concentration of 12.5mg/µl. This indicates that *Morinda lucida* leaves have strong inhibitory effect against *Staphylococcus aureus*. The findings of the *in-vivo* studies showed that the methanol extract was effective against *Staphylococcus aureus* at graded doses. At a lower concentration of 500mg/µl, there was minimal inhibition of *Staphylococcus aureus* infection, while at high concentrations of 1000mg/µl and 2000mg/µl, there was total inhibition of *Staphylococcus aureus* infection This agrees with the findings of Osuntokun and Olajubu [13] which reported that ethanol extract of *Morinda lucida* was effective against wide species of bacteria including *Staphylococcus aureus*. . A similar study demonstrated the efficacy of *Morinda lucida* leaves extract against *Escherichia coli* both *in-vitro* and *in-vivo* [14].

The assessment of haematological parameters showed that the extract has immuno-modulating effect. In the group D, there was decrease in haemoglobin level while the white blood cell total count and neutrophils were increased, which were statistically significant. This is suggestive of leukocytosis induced by *MRSA* infection. The reverse was observed in the groups (A-C) treated with crude methenolic extract of *Morinda lucida* leaves with relatively stable hematological parameters compared to the control.

There was decrease in the values of the lymphocytes of the group D and at low dose of the extract (group A) but at the higher doses of 1000 mg/µl and 2000mg/µl, there was restoration of the values of the lymphocytes compared to the control. This was also reported in a similar study with *Escherichia coli* where the extract also shows immune-stimulatory effects [15]. The histological studies showed that the extract administered at various doses was able to reduce *MRSA* infection damage on the spleen. The inhibitory potentials exhibited by the methanol extract against *Staphylococcus aureus* infection may be due to presence of bioactive properties. These phytochemical components which consist of tannins, alkaloids, saponin, flavonoids may have acted synergistically for the plant to be effective.

The methanol extract of *Morinda lucida* leaves demonstrated dose-dependent efficacy against MRSA infection in albino rats *in-vivo* thus, justifying its use in folk medicine. The extract also inhibited the growth of MRSA *in-vitro* and *in-vivo* as it was able to prevent the establishment of the infection in albino rats. The extract also demonstrated haematopoietic potentials in the experimental rats by increasing their haemoglobin, white blood cell and platelets. In conclusion, the methanol extract of *Morinda lucida* leaves demonstrated stimulatory effects on the experimental animals and also possess bioactive components which could be useful in the treatment of *Staphylococcus aureus* infection.

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Source of Support: Nil, **Conflict of Interest:** None.