IJAMBR 7 (2019) 18-23 ISSN 2053-1818

International Journal of Applied Microbiology and Biotechnology Research

www.bluepenjournals.org/ijambr

Effects of *Irvingia* species leaf extract on *Streptococcus pneumoniae* infected albino rats

doi.org/10.33500/ijambr.2019.07.002

Arikekpar Ibemologi[1]*, Alade Tolulupe[1] and Langley A. Orutugu[2]

[1]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, P. M. B. 071, Bayelsa State, Nigeria.
[2]Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Amassoma, Wilberforce Island, Nigeria.

Article History
Received 23 October, 2018
Received in revised form 10 December, 2018
Accepted 13 December, 2018

Keywords:
Antimicrobial activity, Post-infection *Irvingia* sp., Oral administration, Health benefits.

Article Type:
Full Length Research Article

ABSTRACT

***Streptococcus pneumoniae* causes pneumonia and bacterial meningitis, and bacteremia in children. *Irvingia* sp. or African Bush Mango has nutritional and health benefits and their leaf extracts and other parts have been documented to possess antimicrobial activity, which can be exploited to ameliorate the scourge of antibiotics resistance. In this study, 30 out of 35 albino rats were intraperitoneally challenged with *S. pneumoniae*, followed by oral administration of leaf extract of *Irvingia* sp. The rats exhibited sluggishness, ruffled fur, inappetence and hunch back posture, but recovered from the fourth day after oral administration of *Irvingia* sp. leaf extract. The mean body weights of the rats were 175±1.9 g and 176±1.5 g in the test and the control groups, respectively on the first day of the experiment. The mean body weight of the test group (173±7 g) was lower than the control group (182±40.5 g) on the sixth day of post-infection (P = 0.65). The mean value of food intake by the rats for base line (27.95±5.3g) was higher than the third day (17.35±2.2g) corresponding with inappetence observed in the rats in the test group.**

INTRODUCTION

Streptococcus pneumoniae is a Gram positive cocci, α-hemolytic bacteria (Arora and Arora, 2012) and a facultative anaerobe with virulent strains producing capsular polysaccharide colonies on blood agar, but anaerobic incubation gives off β-haemolysis (Mandell et al., 2007; Cardozo et al*.,* 2006; Arora and Arora, 2012). *S. pneumoniae* is leading causative agent of community acquired pneumonia and can frequently cause localized infections, acute otitis media, pneumonia, meningitis, and bacteremia in humans (Mandell et al., 2007; Hausdorff et al., 2005).

S. pneumoniae can be injected into adult rats intrapulmonarily, infant rats are injected intrabronchially and other methods of inoculation may include intrathoraic and intraperitoneal methods (Candiani et al., 1997; Smith and Abbott, 1994; Alexander et al., 1994). The routes of drug administration to the rats include intravenously (I.V) into their tail veins, subcutaneously (S.C), intramuscularly (I.M), intraperitoneally or orally (Aarde et al., 2013; Hollister-Lock et al., 2013).

Irvingia gabonensis which is also known Bush Mango or African Mango due to its similarity to mango is an economically important tree native to most tropical forests in West and Central Africa (Lowe et al., 2000; Harris et al., 1996). Some uses of *Irvingia* spp. may include, edible purposes, antidiabetics, regulating serum cholesterol levels, weight management, analgesic properties, dysentery treatment, dental care and also as antibacterials and antifungals (Okolo et al., 1995; Ngondi et al., 2005; Ayuk et al., 1999).

The leaf extracts of *I. gabonensis* contain phytochemicals that confers antibiotic activities against

*Corresponding author. E-mail: ariko20002000@yahoo.com.

Table 1. Protocols for *Streptococcus pneumoniae* (SP) injection and treatment with *Irvingia* sp. Paste.

Treatment groups	Treatment
Group 1(control)	Rats were only given feed and water with no SP inoculation and no *Irvingia* administration
Group 2	Each rat was injected with 1 ml of SP with no *Irvingia* extract paste administration
Group 3	Each rat was injected with 1 ml of SP with 50 mg/kg/bw of *Irvingia* extract paste after 24 h
Group 4	Each rat was injected with 1 ml of SP with 100 mg/kg/bw of *Irvingia* extract paste after 24 h
Group 5	Each rat was injected with 1 ml of SP with 250 mg/kg/bw of *Irvingia* extract paste after 24
Group 6	Each rat was injected with 1 ml of SP with 500 mg of *Irvingia* extract paste after 24 h
Group 7	Each rat was injected with 1 ml of SP with Lethal doses of 5500, 6000, 6500, 7000 and 7500 mg of *Irvingia* extract paste respectively for each rat in the group (Ewere et al., 2016)

SP, *Streptococcus pneumoniae* injection; **mg/kgbw,** milligram per kilogram body weight (Ayodele et al., 2015).

Escherichia coli and *Staphylococcus aureus* (George and Zhao, 2007). These constituents include saponin, flavonoids, tannins, cardiac glycoside, anthraquinones and alkaloids (Oluwafemi et al., 2014).

Pneumococci resistance to antibiotics has been well documented by several authors (Appelbaum et al., 1997; Emele, 2000; Doern et al., 2001; Benbachir et al., 2001). Due to the some undesirable side effects caused by orthodox therapies, attention is gradually shifting to plants which have been demostrated as a good source of drugs (Shakya et al., 2010; Samadder and Khuda-Bukhsh, 1994).

MATERIALS AND METHODS

Sample size and sampling technique

A total of 35 adult rats were used for the study by simple random sampling technique. Sample size was determined by Resource equation method (Fitts, 2011).

Plant collection and processing

Fresh leaves *I. gabonensis* were collected from a farm land in Kaiama, Bayelsa State of Nigeria. The leaves of *I. gabonensis* were air-dried under the sun for three (3) weeks and grinded manually with a blender into fine powder, sieved and well packed in a sterile polythene bag. It was stored in a dry place avoiding cold air, water or moisture contact. On extraction, 225 g of the fine grinded leaf were measured out and poured into 350 ml of 80% ethanol. The solution was properly mixed within 3 h and allowed to stand for 48 h at room temperature (25±2°C). After the 48 h of standing, the dissolved solution of powdered leaf and ethanol was filtered by means of filter paper carefully into a clean glass cylinder. The filtrate was then placed in water bath and dried to evaporation by heat (45°C) until the filtrate forms a paste, and stored in sterile containers at 4°C, in the refrigerator for later use (Malann et al., 2014).

Experimental animal collection and design

Thirty-five adult albino rats were housed in netted and aerated decent cages in the animal house of the Department of Medical Laboratory Science. On arrival, the mean ± standard deviation body weights of rats were 127±2.5 g. The rats were fed constantly with growers mash for 28 days and then weighed on Day 1, Day 3 and Day 6 of this experiment. The rats were divided into seven groups of five per group as shown in the Table 1.

Collection and storage bacteria strain

Strain samples were collected from sputum samples at Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria and were confirmed by Optochin sensitivity (Richard et al., 1997) and bile solubility testing, after isolation in 5% sheep blood agar supplemented with 10 mg of gentamycin powder-an antibiotic selective for *S. pneumoniae* growth (Murray et al., 2003).

Harvesting and preparation of inoculum

Colonies of the *S. pneumoniae* were harvested with a sterile wire loop and introduced into 5 ml of Tryptic soy broth and incubated overnight. Organisms were sedimented by centrifugation and mixed with sterile phosphate-buffered saline until they matched the turbidity standard-McFarland Equivalence Turbidity Standards (0.5). A 10 fold serial dilution was done and plated on blood agar plates to ascertain the number of viable bacteria as colony forming unit per ml (Benjamin et al., 2006).

Table 2. Observations from day three to day five.

Group	Treatment	Observation		
		Day 3	Day 4	Day 5
1	No *Irvingia* spp. paste	Normal	Normal	Normal
2	1 ml of SP and no *Irvingia* spp. paste	Abnormal	Abnormal	Abnormal
3	1 ml of SP + 50 mg/kg of *Irvingia* spp. paste	Abnormal	Abnormal	Abnormal
4	1 ml of SP + 100 mg/kg of *Irvingia* spp. paste *Irvingia* spp. paste	Abnorma	Abnormal	Abnormal
5	1 ml of SP + 250 mg/kg of *Irvingia* spp. paste	Abnormal	Abnormal	Abnormal
6	1 ml of SP + 500 mg/kg of *Irvingia* spp. paste	Abnormal	Abnormal	Abnormal
7	1 ml of SP + lethal doses of 5, 500, 6000, 6500, 7000 and 7500 mg/kg of *Irvingia* spp. paste	LDA	LA	LA

Normal, Active, raised fur, normal appetite; **Abnormal,** sluggishness, Inappetance and hunched back posture; **LDA,** lethal dose administered; **LA,** low activity and Inappetance; **SP,** *Streptococcus pneumoniae* injection.

Inoculation of bacterial inoculm

About 1 ml each of the *S. pneumoniae* inoculum was administered into the intraperitoneal cavity of the rats using a 29 wire gauge needle insulin syringe after sterilizing with 70% alcohol and the animals were observed immediately after the injection for any signs of distress or illness (Benjamin et al., 2006).

Irvingia extracts treatment of infected rats

On Day 2, twenty-five rats in the Groups 3-7 were treated with varying doses of leaf extract of *Irvingia* injected intraperitoneally after *S. pneumoniae* injection on Day 1 (Richard et al., 2004; Mukherjee, 2002).

Rat daily body weight and mass of food ingested

The rats body weights were measured every morning and fed from metal cups specifically designed to minimize food spillage; and the daily food intake of each rat was determined directly by weighing the cup just before and after refilling (Darsaud et al., 2003).

Termination of treatments and collection of blood sample

Bacteremia was accessed on Days 4 and 6, as the dorsal veins of rats from all groups were sterilized with 80% ethanol and punctured with sterile lancet, then 0.01 ml was spread on 5% sheep blood agar and incubated at 37°C (Richard et al., 1997, 2004).

Statistical analysis

Data in Table 4 were analyzed with Two Sample T-test and the P-values for the body weights of the test and control rat groups for Days 1, 3 and 6 were 0.23, <0.001 and 0.65, respectively, while that for the food intake in test and control rat groups were 0.72, <0.001 and 0.034.

RESULTS

Twenty four hours after intraperitoneal inoculation of the rats in Groups 2-7 with *S. pneumoniae*, they exhibited Inappetence, ruffled fur, sluggishness and hunched back posture. However, these symptoms were not observed in rats from Group 1 that were not inoculated with *S. pneumoniae*.

Rats in Group 2 showed sluggishness and drastic loss of appetite by Day 3 after they were infected with *S. pneumoniae* on Day 1 but *Irvingia* sp. paste was not orally administered by Day 2. Rats from Groups 3, 4, 5 and 6 were given oral administration of *Irvingia* sp. paste by Day 2 after they were infected with *S. pneumoniae* on Day 1, and they showed reduced sluggishness and lack of appetite compared to those in Group 2 (Table 2).

Results of Days 4 and 5 shows that rats, across Groups 1 to 6 exhibited similar results as shown in Day 3. However, when lethal doses of 5, 500, 6000, 6500, 7000 and 7500 mg/body weight of *Irvingia* sp. paste were administered to different rats in Group 7, rats showed low activity and inappetence.

Observations on Days 6, 7 and 8 from Table 3, showed that Groups 3 to 6 became active with improved appetite. Group 2 also showed improved appetite but low activity compared to the rats in Groups 3-6. On Day 6, rats in Group 7 that had oral administration of 6500 and 7000 mg/kg bodyweight of *Irvingia* sp. paste died.

On Day 1 the mean body weight for test Groups 2-7 was 175±1.9 g and in the control group, it was 176±1.5 g. That for Day 3 was 169±13.3 g for the test groups and 179±2.6 g for control group. While by Day 6, the mean the body weight for the test groups was 173±7 g and in

Table 3. Observations on Days 6, 7 and 8 of the *Streptococcus pneumoniae* infected rats.

Group	Treatment	Observation
1	No *Irvingia* spp. paste	Normal activities, raised fur, normal appetite
2	1 ml of SP and no *Irvingia* spp. paste	Low activity, improved appetite
3	1 ml of SP + 50 mg/kg of *Irvingia* spp. paste	Active and improved appetite
4	1 ml of SP + 100 mg/kg of *Irvingia* spp. paste	Active and improved appetite
5	1 ml of SP + 250 mg/kg of *Irvingia* spp. paste	Active and improved appetite
6	1 ml of SP + 500 mg/kg of *Irvingia* spp. paste	Active and improved appetite
7	1 ml of SP + lethal doses 5500, 6000, 6500, 7000 mg/kg of *Irvingia* spp. paste	Rats injected with 6,500 and 7000 mg/kg died

Table 4. Body weights and food intake in the test rats versus the control group.

Variables	Test groups	Control groups	P
Body weight (g)			
Day 1	175± 1.9	176±1.5	0.23
Day 3	169±13.3	179±2.6	< 0.001
Day 6	173±7.5	182±40.5	0.65
Food intake/day (g)			
Baseline	27.95±5.3	28.74±4.2	0.72
Day 3	17.35±2.2	28.85±0.5	<0.001
Day 6	26.9±1.1	29.10±1.6	0.034

Values are mean ± Standard deviation; **P,** P-value (probability).

the control group recorded 182±40.5 g. The mean baseline food intake for test Groups (2-7) was 27.95±5.3 g and in the control group, it was 28.74±4.2 g. Day 3 rat groups had mean baseline food intake as 17.35±2.2 g for the test groups and 28.85±0.5 g for the control group. While by Day 6, the mean food intake for the test groups was 26.9±1.1 g and in the control group, it was 29.10±1.6 g.

DISCUSSION

The aim of this research was to ascertain the antimicrobial activity of *Irvingia* leaf extract against *S. pneumoniae* infected rats, and in a related research on *Irvingia* antimicrobial activities against two common pathogens, *E. coli a*nd *S. aureus*, findings revealed that the plant contains chemical constituents which posses antibacterial activity against both bacteria (Nworie et al., 2016).

In this study, the inoculation of *S. pneumoniae* into the healthy adult rats caused the manifestation of various clinical signs of pneumonia such as Inappetence, sluggishness, ruffled hair and hunch back. However, the treatment of pneumonia in the rats with varying doses of *Irvingia* leaf extract shows a significant effect on the infected rats as many of the observed signs were reversed 3-4 days after *Irvingia* treatment. In a related research on the antibacterial activity of hot and cold water and ethanolic extract of the leaf and stem bark of *I. gabonensis. E. coli* and *S. aureus* were susceptible to all the extracts (Kuete et al., 2007).

Findings from this study showed that the mean food intake of rats in the test group reduced from 27.95 to 17.35 g by Day 3 as a result of lack of appetite. On Day 1, the mean bodyweight for rats in the test group was 175±1.9 g and 176±1.5 g in the control group. At Day 3, it was 169±13.3 g for test group and 179±2.6 g for control group. On Day 6, the mean body weight for test group was 173±7 g and 182±40.5 g in the control group. This reveals weight loss in the test group between the Day 1 to Day 6 when the rats started recuperating (6±11.4 to 4±6.3 g). However there is weight increase observed in the control group, which may occur in the test group when kept for about 12 weeks after infection as observed by Fatemeh et al. (2017).

Some other plant extracts reported to show antimicrobial activities in challenged rats is the *Cnidoscolus aconitifolius* plant extract, which indicated broad spectrum antibacterial activity against *S. aureus, Shigella* spp., *Salmonella* spp. and *S. pneumoniae* (Ekeleme et al., 2013).

In another report, aqueous extract of *Vitelleria paradoxa* was shown to clear Salmonellosis within twelve days in previously infected rats via single dose oral administration of *Salmonella typhimurium* (Fodouop et al., 2017). However in this study the rats recovered from the fourth day after oral administration of the *Irvingia* sp. extract. The lethal doses of *Irvingia* from this research shows that doses higher than 5500 mg/kg of the paste extract administered to the infected rats causes death, and a recent research on *Spondias mombin* effect on rat toxicity which contains similar phytochemicals like *Irvingia* spp. closely supports this outcome with a lethal dose above 5000 mg/kg (Muhammad, 2015; Ewere et al., 2016).

Conclusion

The abundance of the bush mango plant in West and Central Africa should be exploited research-wise because its leaf extract confers antimicrobial activity against *Streptococcus pneumoniae* and other microbes. The observation is interesting because the plant grows more in the African continent and probably why scientific publications about the plant are still a bit limited. Thus, further research on the bush mango should be aggressively embarked upon, as it may aid in the reduction in the drug resistance, cost of treatment of the disease, economic and research growth/development in the region.

Compliance with ethics and guidelines

Animals were handled according to the APA Committee on Animal Research and Ethics (CARE).

Conflict of interest

Authors have no conflict of Interest.

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