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Full Length Research

Evaluation of wound healing potential of *Jatropha curcas* leaf extracts ointment based on wound infection in Wistar rats model

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ABSTRACT: This research study was conducted to evaluate the wound healing potential of ointment formulated with *Jatropha curcas* leaf extracts based on wistrar rat model. The therapeutic activities of the plant extracts were determined by topical application of the herbal ointment. Wound healing rate took place on the 16th day of post treatment with 1.0 g of *J. curcas* ointment formulation and 12th day with 2.0 g of the ointment, was similar compared with standard drug Gentamicin ointment (12 days). The control untreated group (62.3±0.51%) rate of wound closure observed persisted beyond the 19th day of post-wounding. The haematological parameters of the infected rats treated with herbal ointment and the control group untreated were not significant different ($p>0.05$). Significant increase in white blood cell (WBC) count in untreated group was recorded when compared with the treated group. However, treatment with the formulated ointments significantly increase the elevated WBC count in the treated group. The toxicological parameters in the serum of the rats are useful makers for the assessment of tissue damage. The serum Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activities in the treated and untreated experimental groups were not significantly ($p>0.05$) different. The phytochemical components found in *J. curcas* leaves contain some useful potential antimicrobial agents that possess wound healing properties when formulated into a topical ointment for topical application.

Keywords: Herbal ointment, *J. curcas* leaf extracts, *Staphylococcus aureus,* wound healing.

INTRODUCTION

Staphylococcus aureus is a major human pathogen that has been linked to a wide range of infections around the world. *S. aureus* causes significant epidemiologic and therapeutic issues in Nigeria. Nigeria, the most densely populated African country, has seen an increase in both community-acquired (CA) and hospital-acquired (HA) *S. aureus* over the last 20 years, while antibiotic treatment has been hampered by the spread of *S. aureus* strains resistant to multiple antibiotics, including methicillin (Harbarth *et al*., 2005; Hallin *et al*., 2008).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major threat to public health in many parts of the world, causing significant morbidity and mortality. MRSA infections and outbreaks have become more common in recent years. MRSA is frequently multidrug resistant, and treatment options are limited. MRSA is typically multidrug-resistant, making it resistant to beta lactams, aminoglycosides, fluoroquinolones, and macrolides. To address this issue, there is an urgent need to develop anti-MRSA agents with novel mechanisms of action. With the increasing prevalence of antibiotic-resistant infections, an arsenal of either new agents or antibiotic supplementation is required. Plant-based natural products could be interesting alternatives (Alzohairy, 2011).

Staphylococcus aureus is recognized as one of the major causes of infection in humans occurring in both the community and the hospital. Multidrug resistant staphylococci have become a major nosocomial pathogen (Nascimento *et al*., 2010).

The use of medicinal plants for the treatment of various infections in traditional communities has been a long-standing global practice. It is estimated that herbal regimens are used by 80% of the African population for disease treatment and control (Hugo and Russell, 2012). This justifies the investigation of medicinal plant extracts as a potential source of alternative infection therapy. The medicinal plant's pharmacological properties are immense; plant-based remedies play an important role in the health of millions of people, particularly in rural areas. The current problems associated with antibiotic use have rekindled interest in plants that have antimicrobial properties. Herbal medicine remains the primary source of care for 75 to 80% of the world's population in developing countries due to its greater cultural acceptability and fewer side effects (Aiyegoro and Okoh, 2014).

Jatropha curcas L., also known as physic nut, is a small tree or bush that can reach a height of 5 meters. It belongs to the *Euphorbiaceae* family and has over 170 species identified. Jatropha, a drought-resistant shrub or tree, is abundant in semi-cultivated or wild areas of Central and South America, Africa, India, and Southeast Asia (Heller,2013). It is a drought-resistant, perennial plant with numerous applications, and it is becoming increasingly important in the production of biodiesel. It has lush, attractive branchlets. The tree has a straight trunk with large white patches covering the grey or reddish bark. It has green leaves with 5 to 7 cm long and 6 to 15 cm wide lobes. The branches' yellowish latex is present, resulting in dark stains. Terminal inflorescences form on branches. The plant is monoecious and the bloom is unisexual (Okoli *et al*., 2008). After pollination, a trilocular ellipsoidal fruit develops. On average, ripe Jatropha fruits are 18 mm long and 10 mm wide, with black seeds (Nath and Dutta 2012). It is a versatile species with a diverse set of characteristics and enormous potential.

The fruit and wood of J*atropha* can be used for a variety of purposes, including fuel. Gum disease, arthritis, gout, jaundice, toothaches, dermatomucosal disorders, bleeding gums, diarrhea, and pyorrhea are all treated with it. A plant extract that has been used to treat leprosy, leucoderma, scabies, small pox, inflammation, allergies, burns, cuts, and wounds (Nath and Dutta 2012). Branch water extract is used to treat tumors, HIV, and wounds. The plant contains organic acids, cyclic triterpene stigmasterol, curcacycline A, Curcin, a lectin, phorbol esters esterases, sitosterol, and its d-glucoside. The leaf and bark have been shown to contain steroidal sapogenins, glycosides, tannins, phytosterols, flavanoids, and phytosterols (Okoli *et al.,* 2008).

Therefore, the importance of identifying new effective antibacterial cannot be overemphasized. In order to find new therapeutic agents, plants that have antibacterial activity have attracted attention in improving health and fitness through the use of more natural products. The Screening of *Jatropha curcas* plants leaf extracts for phytochemicals, antibacterial and wound healing activity is important for finding potential new compounds for therapeutic use.

MATERIALS AND METHODS

Collection and Identification of plants

Jatropha curcas plant leaves samples were collected in August, 2017 from Minna and Zungeru in Niger State, Nigeria .The taxonomic identification were confirmed and authenticated by a Botanist, Mrs G.E Ugbabe, with voucher No. VD 4675 of the Herbarium Department, National Institute for Pharmaceutical Research and Development, Idu Abuja, Nigeria.

Preparation of plant material

Fresh plants of *J. curcas* were harvested and dried under shade at room temperature for a period of ten days. The dried plants were homogenized each by using the laboratory mortar and pestle then finally into powdered form by electrical blender machine.

Extraction process

One hundred and fifty grams (150 g) of the plants powder leaves were macerated in 850 ml of methanol and shaken twice daily for seven days using the modified Chika *et al*. (2007) method. The extracts were filtered using muslin cloth, and the filtrates were dried on a water bath. For each plant, paste-like semi residue was obtained and weighed before being stored in the refrigerator at 40°C until needed.

Phytochemical screening of the plant extract

Basic phytochemical screening which consists of performing simple chemical test to detect the presence of alkaloids, tannins, saponins, anthranoids, glycosides, anthraquinone, steroids were carried in the Department of Biochemistry of Federal University of Technology Minna, following the standard laboratory techniques (Harbone,1994; Trease and Evans, 2004).

Sources of test organisms

A total of 248 swab samples for the screening were obtained from clinical sample of wound, ear, urine, sputum,

Nasal swab, HVS and stool from selected public hospital in Minna Niger State Nigeria.

Biochemical identification

Gram staining reaction was performed on suspected colony from the culture plate according to standard procedures of Cheesbrough (2002). Biochemical tests of catalase and coagulase were performed on isolates to indicate positive case for *Staphylococcus aureus*.

Selective detection of *S. aureus* (mannitol salt agar)

Mannitol salt agar is used in microbial limit tests and for isolating staphylococci from clinical specimens and cosmetics. Chapman developed this formulation to distinguish coagulase positive staphylococci (e.g., *Staphylococcus aureus*) from coagulase negative staphylococci through the formation of golden yellow coloration. The media was prepared in accordance with the manufacturer's instructions (Bannerman, 2009).

Identification of methicilin resistant *Staphylococcus aureus* (MRSA)

Oxacillin resistance screening agar base (ORSB) and its oxacillin supplements (SR0195E) (oxoid England) were prepared per the manufacturer's instructions. A loopful of a 3 hours *S. aureus* culture from Nutrient broth was inoculated on the medium and incubated for 24 hours at 370C. The appearance of blue colony growth indicates a positive case of Methicillin resistant Staphylococcus aureus resistance (Daniel and Abalaka, 2012).

Standardisation of the test organisms

A loopful of the culture of the organisms was inoculated into 5 ml of sterile nutrient broth and incubated for 24 hours. Exactly 0.2 ml of overnight culture of the organism was inoculated into 20 ml of sterile nutrient broth and incubated for 2 to 3 hours. The turbidity of the culture was compared with that of 0.5 Mac-Farland to standardize the culture to 10^6 cfu/m (Daniyan and Abalaka, 2012).

Determination of antibacterial activity

Antibacterial activity was carried out using well diffusion method of Daniyan and Abalaka (2012) as modified. Fresh culture of 3 to 4 hours *S. aureus* was inoculated on the surface of Muller Hinton agar plates. Holes (6 mm) were bored on the surface of the agar and filled with 30 µl of each plant extracts. Gentamicin was used as a positive control and tested on the organisms under same conditions. An average of three replicates for each extracts were used while the antibacterial activities were assessed by measuring diameter zone of inhibition around the well.

Determination of MIC and MBC

The extracts' Minimum Inhibitory Concentration (MIC) on the isolates was determined using microbroth dilution techniques in accordance with the Clinical and Laboratory Standard Institute's recommendations (CLSI, 2009). The MBC values were calculated by removing a loopful of bacterial suspension from the MIC tubes that did not show any growth and sub-culture into nutrient agar plates. The plates were incubated for 24 hours at 370°C. The concentration at which no visible growth was observed after incubation was recorded as the MBC.

***In vivo* study**

Fifteen witsar albino rats of both sexes weighing about 116 to 200 g were used. They were kept in the Biochemistry Laboratory of Federal University of Technology Minna, Niger State, Nigeria. They were fed with normal feeds for two weeks to acclimatize before starting the experiment. The animals were handled according to Canadian Council on animal care (CCAC) guideline on animal use protocol review (1997). The ethical clearance with reference number REF/FUT/PS/PCL/BC/26 was approved before the commencement of the study.

Wound creation

The animals were sedated with chloroform using the open mast method. All surgical interventions were performed under the sterile conditions of general anaesthesia. By removing hairs with a razor, the predetermined area for wound infliction at the back of the animal was prepared for surgery. The albino rats' backs were slashed with a 5 cm excision wound (Daniyan and Abalaka, 2012).

Drug administration

The topical application of formulated extracts ointment on the wound area was used as route of administration which was done daily after cleaning with sterile surgical cotton wool (Daniyan and Abalaka, 2012).

Wound healing activity

Wound contraction, which contributes to closure, is expressed as a percentage reduction in the original wound

size, which was studied from the day of operation until the day of complete epithelisation and evaluated to calculate the degree of wound healing.

Collection of the specimen (blood) from albino rats

After about 17 days of topical administration of the extracts to the excision wound. Following that, blood samples were drawn from them by using syringe and needle placed in a sterile specimen bottle for haematological and toxicological parameters (Daniyan and Abalaka, 2012).

Statistical Analysis

The data were analyzed using one way analysis of varience (ANOVA). Differences in the means between the paired observations were accepted as significant at $p \leq 0.05$.

RESULTS

The preliminary phytochemical screening of methanolic extracts of *J. curcas* revealed the presence of several phtytochemicals as shown in Table 1. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of this study revealed that *J. curcas* plant extracts against *S. aureus* was dependant on the concentration and dosage of the extracts as shown in Table 2.

The result of wound healing activity of this study indicated that complete wound healing rate took place on the 16th day of post wounding with 1.0 g of *J. curcas* ointment formulation and 12th day with 2.0 g of the ointment, as same compared with standard drug Gentamicin ointment (12 days). The untreated group (control) (62.3±0.51%) rate of wound closure observed persisted beyond the 19th day of post wounding as shown in Table 3.

The result of the haematological parameters of wistar rats infected with Methicillin resistant *S. aureus* (MRSA) with *J. curcas* formulated ointments were not significantly different. However, treatments with the formulated ointments significantly ($p<0.05$) decrease the elevated WBC when compared with (control) untreated rats (Table 4.).

The result of toxicological parameters obtained from this study indicated that serum Aspartate Transaminase (AST) and Alanine Transaminase (ALT) activities in infected wistar rat and those treated with *J. curcas* formulated ointments were not significantly ($p>0.05$) different (Figures 1 and 2). However, a significant increase in the serum Alkaline Phosphatase (ALP) activity was observed in *J. curcas* formulated ointments (Figure 3).

Table 1. Phytochemical constituents of crude methanol leaf extracts of *Jatropha curcas* medicinal Plants

Phytochemical components	*Jatropha curcas*
Saponin	+
Anthraquinone	+
Tannins	+
Alkaloid	+
Flavonoids	-
Cardiac glycosides	+
Phlobatanins	+
Steroids	+

Key: + = Present; - = Absent.

DISCUSSION

The antimicrobial susceptibility test results provide important information about the antimicrobial status of the medicinal plant. The antibacterial activities of *Jatropha curcas* leaf extracts were revealed in this study by their zone of inhibition when inoculated with methicillin-resistant *S. aureus* (MRSA). Surprisingly, the plant extracts have antibacterial effects that are dose dependent. Despite the fact that all of the extracts were more active at test concentrations of 120 and 160 mg/ml. Aderogba (2006) reported that higher concentrations of antimicrobial substance inhibited bacterial growth significantly. This was consistent with the findings of this study, as increasing the concentration of all plant extracts resulted in an increase in the zones of inhibition.

All of the plant extracts in this study had the same minimum inhibitory concentration (MIC) of 40 mg/ml. The Minimum Bactericidal Concentration (MBC) is the lowest antimicrobial agent concentration required to kill 99.9% of the initial inoculums (Parish and Davidson, 1993). According to Atangwho (2009), MBC results obtained by plating various dilutions of extract are more reliable than MIC results obtained by using turbidity as an index in assessing antimicrobial activities of plant extracts. However, except for the methanol leaf extract of *J. curcas*, which had lower MBC (20 mg/ml), the MBC results in this study followed the same patterns as their corresponding MIC. The lower MBC validates the higher antimicrobial activities even more (Table 2). *J. curcas* has previously been shown to be effective in wound healing in rats. The effects of these plants on MRSA-infected wounds were investigated in this study. The formulated herbal ointments of *J. curcas* 2.0 and the standard drug (Gentamicin ointment) exhibit comparable activities, with 100% wound closure on the 12th post-wounding day. This suggests that *J. curcas* formulated herbal ointments are as promising as Gentamicin in terms of providing faster wound healing when applied to wounds. The phytochemicals found in the selected plants are known to aid in wound healing, owing to their astringent and antimicrobial properties, which appear to be responsible for rapid cellular proliferation,

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *J. curcas* plant extracts against *S. aureus*.

Conc.(mg/ml)	MIC		MBC	
	J. curcas (1.0 g)	*J. curcas (2.0 g)*	*J. curcas (1.0 g)*	*J. curcas (2.0 g)*
160	-	-	-	-
120	-	-	-	-
80	-	-	-	-
40	-	-	-	-
20	-	+	+	+
10	+	+	+	+
5	+	+	+	+

Key: +: Growth detected; -: No growth.

Table 3. Rate of wound healing caused by methanolic leaf extracts of formulated herbal ointments on MRSA infected wound in rats.

Ointments	Time (Days)					
	3	7	9	12	16	19
Untreated	11.34±0.93	12.10±0.28	22.36±0.11	38.16±0.09	51.50±0.18	62.35±0.51
J. curcas (1.0 g)	13.10±0.28	20.22±0.28	59.22±0.09	86.99±0.08	100.00±0.00	
J. curcas (2.0 g)	12.34±0.34	15.20±0.25	28.07±0.91	100.0±0.0		
Gentamicin (Standard drug)	25.14±0.43	56.32±0.11	87.67±0.09	100.0±0.0		

Table 4. Activity of methanol leaf extracts of on haematological parameters of rats infected with Methicillin resistant *S. aureus* (MRSA).

Parameters	*J. curcas* (1.0 g)	*J. curcas* (2.0 g)	Gentamicin	Control (untreated)
WBC ($x10^9$ /L)	2.80±0.54 ^{a}	2.50±0.34 ^{a}	2.50±0.11 ^{a}	3.6.10±0.09^b
RBC ($x10^{12}$ /L)	5.54±0.21 ^{a}	5.32±0.67 ^{a}	5.44±0.11 ^{a}	5.41±0.23^a
PCV (%)	44.6±0.11^a	43.66±0.11^a	45.45±0.11^a	44.21±0.05^a
HGB (g/dL)	14.86±2.07^a	14.55±1.08^a	15.15±1.07^a	14.73±1.00^a
MCV (FL)	77.05±3.41^a	72.05±5.12^a	77.92±3.21^a	74.89±2.17^a

Key: WBC = White Blood Cells, RBC = Red Blood Cells, HGB =Hemoglobin, MCV =Mean Corpsular Volume, PCV= Pack Cell Volume. Superscript a = No significant difference b = significant differences occur.

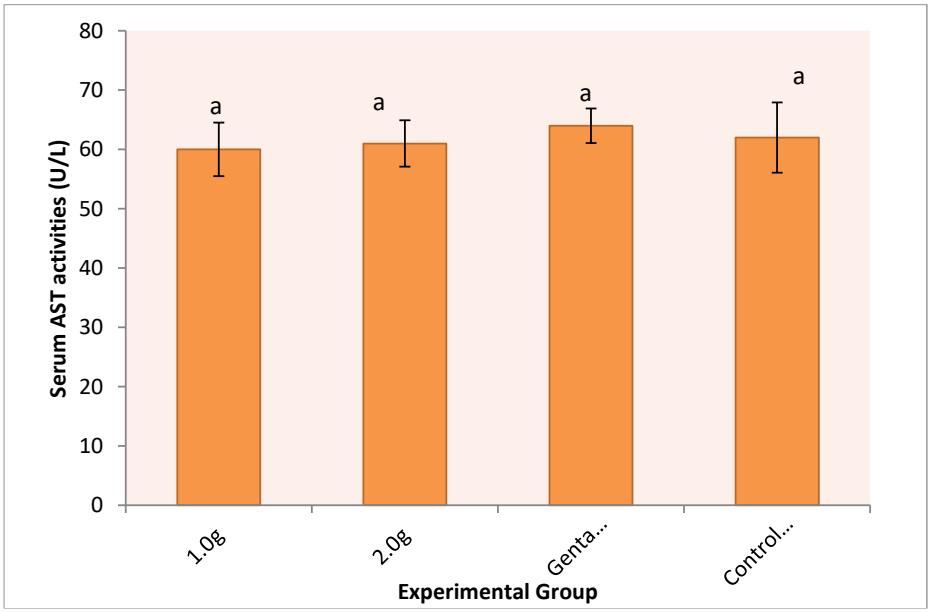

Figure 1. Serum Aspartate Transaminase activitiy of infected rats treated with *J. curcas.*

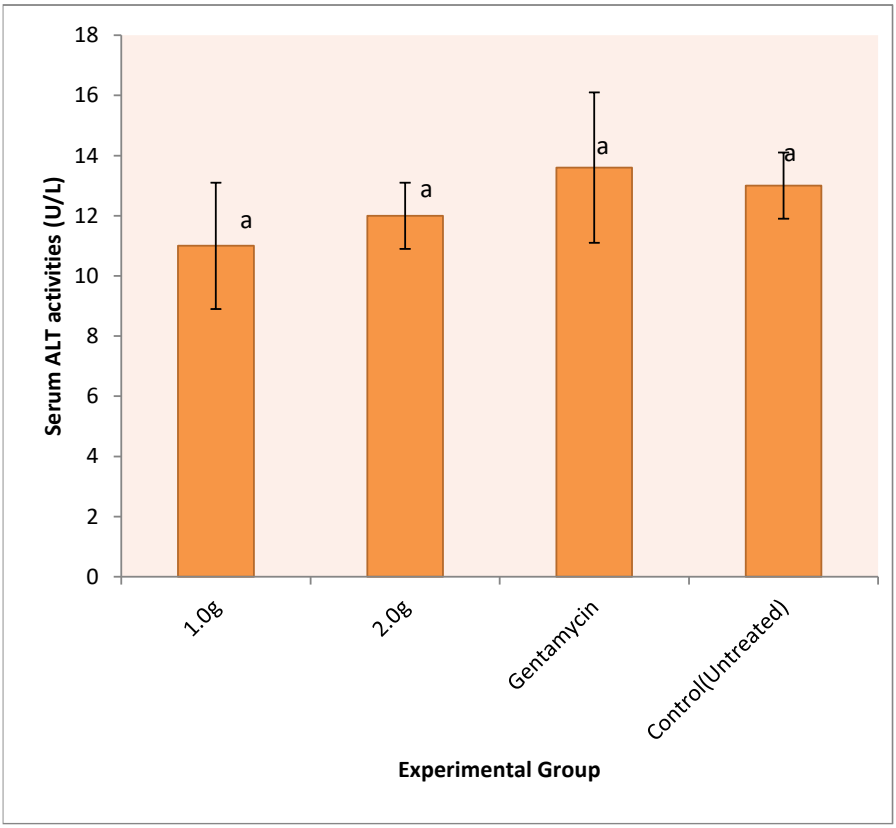

Figure 2. Serum Alanine Transminase activitiy of infected rats treated with *J. curcas.*

collagen deposition, epithelialization, myofibroblast production, and angiogenesis around the injured area (Udupa *et al*., 2006).

The very good healing observed with *J. curcas* formulated ointments could be attributed to the synergistic effects of the phytochemicals components of the plants. Although the negative control group was left untreated, the wound healing observed with negative control group (62.35±0.18% wound closure) was probably due to natural response to wound as the body immune system and blood clot factors set to play and bring about wound healing without the aid of any wound healing agents. Similarly, this study agrees with earlier report on wound healing properties of methanolic extracts of *Gossypium barbadense* leaves by (Ikobi *et al*., 2012).

Plant products are potential wound healing agents that are widely used due to their widespread availability, non-toxicity, lack of undesirable side effects, and efficacy as crude preparations (Kodati *et al*., 2011). As a result, the effects of the ointments on toxicological and haematological parameters were investigated in order to further validate the safety of these plant-formulated ointments for clinical application.

The toxicological parameters measured in rat serum are useful 'markers' for assessing tissue damage. The measurement of various enzyme activities in body fluids is important in disease investigation and diagnosis (Bashir *et al*., 2015), as well as the assault on organs/tissues and, to a lesser extent, the toxicity of test plant extracts (Shittu *et al*., 2015a). The transaminase (ALT and AST) are 'markers' of liver damage and can thus be used to assess liver cytolysis with Alanine Transaminase (ALT) being a more sensitive biomarker of hepatotoxicity than Aspartate Transaminase (AST) (Yakubu and Musa, 2012). Consequently, in this research work the serum ALT and AST of MRSA infected rats treated with the plant formulated ointments were not significantly altered, this shows that these plant extracts did not cause inhibition/activation of these enzymes.

Alkaline phosphatase is a plasma membrane and endoplasmic reticulum "marker" enzyme. It is frequently used to evaluate the integrity of the plasma membrane and the endoplasmic reticulum (Salau *et al*., 2013). The increased serum ALP activity in rats treated with *J. curcas* formulated ointments could be due to enzyme activation or an increase in the rate of enzyme synthesis induced by the constituents of the plant extracts in the formulation. This increase may also indicate increased functional activity of the organs, as the rate of ion transport across cell membranes in these tissues may increase (Lawal *et al*., 2015a). Total protein concentrations are useful 'markers' of the liver and kidney's secretory, synthetic, and excretory functions (Yakubu *et al*., 2003). The lack of change in total protein levels suggests that the liver's ability to synthesize

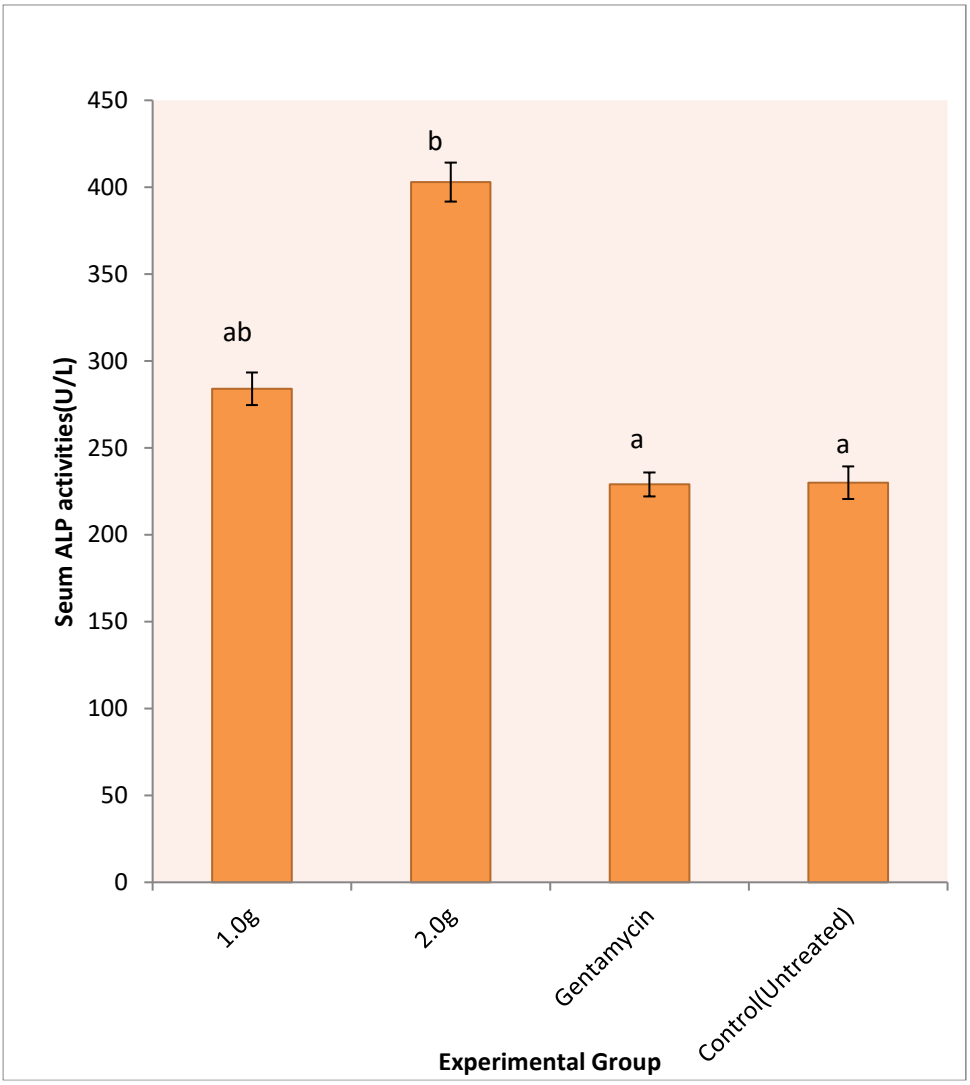

Figure 3. Serum Alkaline Phosphatase (ALP) activitiy of infected rats treated with *J. curcas.*

proteins has not been compromised.

The examination of the numbers and morphology of the cellular elements of the blood, the red cells (erythrocytes), white cells (leucocytes), and platelets (thrombocytes), provides the opportunity to investigate the presence of several metabolites and other constituents in the body of animals and plays a vital role in the physiological, nutritional, and pathological status of an organism (Lawal *et al.,* 2015b). This study found that untreated MRSA infected rats had lower Pack Cell Volume (PCV), Red Blood Cell (RBC), and Hemoglobin (HGB). These findings indicated that MRSA infection in rats does not result in anaemia. The significant increase in PCV, RBC, and HGB observed in rats treated with *J. curcas* formulated ointments when compared to control rats and other experimental groups, however, reflects the extract's erythropoietic activities (Lawal *et al*., 2015b) (Table 4).

White blood cells protect the body from infections and foreign bodies. The significant increase in WBC count observed in MRSA-infected rats that were not treated may indicate an immunological response by the animals to the *S. aureus*, which augmented the production of more WBC, thereby improving the animals' health (Bashir *et al*., 2015). Treatments with *J. curcas*. formulated herbal ointments, on the other hand, significantly reduce the elevated WBC when compared to untreated rats.

Conclusion

This study has shown that the methanol leaf extracts of *J. curcas* contains some useful potential antimicrobial phytochemical that are inhibitory to MRSA. However, ointment formulated with higher dose of 2.0 g *J. curcas* alone give a better wound healing effect comparable with the standard drug. The formulated ointments were found

not to cause significant alteration to the normal level of biochemical and haematological parameters in rats. Therefore, *J. curcas* may be considered as a natural and source of antimicrobial for therapeutic purposes.

CONFLICT OF INTREST

The authors declare no conflict of interest exist.

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