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in vivo antibacterial and therapeutic properties of *P. ostreatus* against *Staphylococcus aureus*

***Oyekanmi, B. A.[1], Onifade, A. K.[2], Osho, I. B.[3], Ajayi O. T.[4]**

Abstract

Objectives: The use of natural products is embraced by a larger percentage of the world population. Most species of fungi including mushrooms produce useful secondary metabolites that stimulate the immune system against infection and diseases. Investigations were conducted to assay the therapeutic potentials of *P. ostreatus* against pathogenic staph infection.

Methods: The methanol extract of *P. ostreatus* was prepared using the cold extraction method. Thirty Wistar albino rats weighing 82.0 g to 99.2 g were distributed into 6 groups of 5 and inoculated orally with actively growing *Staphylococcus aureus* suspension. *P. ostreatus* methanol extract $LD_{50} > 5000$ mg/kg was used to determine the graded doses for the study. Graded doses of the extract 625 mg, 1250 mg, and 2500 mg were administered orally to the experimental animals for seven days.

Results: The negative control and 625 mg had skin ulceration while 1250 mg to 2500 mg produced apparently healthy skin. Bacterial count after 7 days post-treatment was significantly high in the negative control and 625 mg dose ($32.00 \times 10^4 \pm 6.10^b$; $43.40 \times 10^4 \pm 6.20^b$ Cfu/ml) $P < 0.05$. Haematological and serum biochemical values were not significantly $P < 0.05$ affected. *Pleurotus ostreatus* administration at 1250 to 2500 mg produced a statistically low colony count that was comparable with 13.33 mg Ciprofloxacin and placebo.

Conclusion: *Pleurotus ostreatus* at 1250 to 2500 mg was able to produce clinical recovery from *S. aureus* infection while 625 mg could not. The extract had no deleterious effect on the blood parameters, liver enzymes, and kidney biomarkers.

Keywords: *Pleurotus ostreatus*, anti-staphylococcal, haematological, biochemical

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propriétés antibactériennes et thérapeutiques in vivo de P. ostreatus contre Staphylococcus aureus

***Oyekanmi, B. A. [1], Onifade, A. K. [2], Osho, I. B. [3], Ajayi O. T. [4]**

Resume

Objectifs: L'utilisation de produits naturels est adoptée par un plus grand pourcentage de la population mondiale. La plupart des espèces de champignons, y compris les champignons, produisent des métabolites secondaires utiles qui stimulent le système immunitaire contre les infections et les maladies. Des recherches ont été menées pour évaluer les potentiels thérapeutiques de P. ostreatus contre l'infection staphylococcique pathogène.

Méthodes: L'extrait au méthanol de P. ostreatus a été préparé en utilisant la méthode d'extraction à froid. Trente rats albinos Wistar pesant de 82,0 g à 99,2 g ont été répartis en 6 groupes de 5 et inoculés par voie orale avec une suspension de Staphylococcus aureus en croissance active. Un extrait méthanolique de P. ostreatus DL 50 > 5000 mg/kg a été utilisé pour déterminer les doses graduées pour l'étude. Des doses graduées de l'extrait de 625 mg, 1250 mg et 2500 mg ont été administrées par voie orale aux animaux de laboratoire pendant sept jours.

Résultats: Le témoin négatif et 625 mg présentaient une ulcération cutanée tandis que 1250 mg à 2500 mg produisaient une peau apparemment saine. La numération bactérienne après 7 jours post-traitement était significativement élevée dans le contrôle négatif et la dose de 625 mg (32,00 x 104 ± 6,10b ; 43,40 x 104 ± 6,20b Cfu/ml) $P < 0,05$. Les valeurs hématologiques et biochimiques sériques n'étaient pas significativement affectées par $P < 0,05$. L'administration de Pleurotus ostreatus à raison de 1250 à 2500 mg a produit un nombre de colonies statistiquement faible qui était comparable à 13,33 mg de ciprofloxacine et à un placebo.

Conclusion: Pleurotus ostreatus à 1250 à 2500 mg était capable de produire une guérison clinique de l'infection à S. aureus alors que 625 mg ne le pouvaient pas. L'extrait n'a eu aucun effet délétère sur les paramètres sanguins, les enzymes hépatiques et les biomarqueurs rénaux.

Mots clés: Pleurotus ostreatus, antistaphylococcique, hématologique, biochimique

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INTRODUCTION

Antimicrobial resistance and epidemics of infections from unknown origin necessitate a continuous search for drugs and antimicrobials from natural sources. It is therefore important to fortify and strengthen the immune system to face future health challenges of outbreaks of infectious and life-threatening diseases. *Staphylococcus aureus* causes boils, cross infections of wounds, ulcers, burns, and septicemia. Extracellular enzymes produced by *S. aureus* contribute to its invasiveness and pathogenicity (1). There are resistant strains of *Staphylococcus aureus* such as methicillin-resistant *S. aureus*. These strains are resistant to Methicillin, related penicillin, and other common antibiotics. They are causative agents of hospital infections, especially wound infections and septicemia (1).

Mushrooms have been found useful as a food source and traditional medicines around the world including Japan, China, and Nigeria. Edible mushrooms are known to be safe and devoid of adverse side effects. Therapeutically fungi are of specific interest because they are eukaryotic organisms and their metabolism is closely related to man and animals. Mushrooms are macro-fungi and are now widely accepted as an untapped source of potentially powerful natural products of pharmacological significance (2). Various bioactive compounds extracted from mushrooms fruiting bodies, cultured mycelia and cultured broth are polysaccharides, glycosides, proteins, flavonoids and carotenoids (3). Most of these compounds function as Biological response modifiers (BRM) which have been shown to enhance various immune responses (4).

In recent years chemists and immunologists have discovered the importance of the polysaccharides inherent in mushrooms. Studies of fungi have shown that the Basidiomycetes and Ascomycetes divisions are an immense source of biologically active components, but few species were tested for therapeutic importance (5). *Pleuroteus ostreatus* is a gilled mushroom commonly known as milky or Oyster mushroom and one of the mostly consumed mushrooms. It is of Division: Basidiomycota, Class: Agaricomycota and order: Agaricales. Records of health-promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol-lowering and immunostimulatory effects have been reported for some species of mushrooms (6). The *in vivo* antibacterial activity and effect of methanol extract of *P. ostreatus* on haematological parameters and serum enzymes after 7 days oral administration, against *S. aureus* inoculated Wistar albino rats were investigated in this study.

MATERIALS AND METHODS

Collection and Identification of Mushroom Species:

Fresh fruiting bodies of *Pleurotus ostreatus* were obtained from a commercial farm at latitude 07° 40 North and longitude 04° 30 East, Osun State in Nigeria. The fully matured mushrooms were packed in a polyethene bag, labelled and transported to the microbiology laboratory within 24 h of collection. The mushroom was identified by a Botanist. Macroscopic identification was based on colour, odour, and spore print and morphological characteristics. The mushroom was further identified based on gene sequences using molecular characterization. The Oyster mushroom used for the study was 99.09 % organism matched of *Pleurotus ostreatus* strain PAsp14 (7).

Preparation of Extracts

The mushrooms were dried at ambient temperature for 2 weeks and processed using a cold extraction method as described by Oyekanmi *et al.* (7). The mushroom powder was soaked at the ratio of 1:10 of ground powder to 98 % Methanol. The mouth of the beaker was covered with foil paper to prevent evaporation and the mixture was allowed to stand at room temperature (28 ° C to 30 ° C) for 48 h with frequent agitation. The supernatant was filtered using a muslin cloth and filtrate was concentrated *in vacuo* in the rotary evaporator at 40 °C. The extract was evaporated to dryness in small vials at 40 °C in the laboratory oven (Lab-Tech). A stock solution of 500 mg/ml concentration was prepared using sterile physiological saline (0.85 %) as diluent and sterilized using 0.45 mm diameter membrane filter before use. Concentrations required were prepared from the stock using RV/O where R = required concentration, V = required volume, and O = original concentration. They were labelled and preserved at 4 °C.

Collection of Bacterial Isolate

Clinical isolate of Staphylococcus aureus was obtained from Federal Medical Centre, Owo, Nigeria.

Preparation of inoculums

The bacterial species was isolated from the

wound sample and obtained on agar slant, labelled, and preserved at 4 °C. The isolate was sub-cultured on Manitol salt agar and incubated at 37 °C for 24 h. The bacterial growth was confirmed using gram staining procedures and biochemical tests. A broth culture of actively growing bacteria was prepared in peptone water. The turbidity of 24 h actively growing bacteria suspension was adjusted to obtain turbidity optically comparable to that of 0.5 Mc Farland standards (10^6 Cfu/ml). Each of the experimental animals was inoculated with 0.3 ml of the bacterial (*S. aureus*) suspension.

***Management of* Experimental Animals**

Thirty (30) apparently healthy Wistar albino rats of body weight 82.0 g to 92.2 g were obtained from the animal house of the College of Health Sciences, Osun State University Osogbo. The animals were housed in plastic cages and fed with standard rat feed once a day and clean drinking water. The beddings were laid with wood powder and changed daily. The room temperature was maintained and the light was 12 h light and 12 h darkness. Animal maintenance, use, and care was according to the National Institute of Health (NIH) animal care guidelines (8).

Experimental Design

The experiment was conducted in a Completely Randomized Design (CRD). The Wistar rats were distributed into 6 groups of 5 and allowed to acclimatize in 7 days. The cages were properly labelled and each rat was also marked employing different dyes, on the tail for proper identification. Experimental animals were inoculated orally with 0.3 ml *S. aureus* bacterial suspension of standard inoculums ($1x10^6$ Cfu/ml) and allowed for 3 days incubation period. The graded dose of extract administered was determined using $LD_{50} > 5000$ mg/kg (5). A graded dose 625 mg (low), 1250 mg (moderate) and 2500 mg/kg body weight (high) was administered for 7 consecutive days. The animals were nurtured for another 7 days with feed and drinking water only. After 7 days post-treatment the animals were starved for 12 h, sacrificed using cervical dislocation method, and the blood sample was collected from the heart. Controls were set up which include: Control 1- Normal control (placebo): not inoculated but administered with 0.85 % NaCl (Sodium chloride solution); Control 2- Positive control: Inoculated and administered with 13.33 mg/kg body weight ciprofloxacin capsule; Control 3-Negative control: Inoculated with *S. aureus* but not administered with antibacterial. Groups 4, 5, and 6 were the test groups, inoculated and administered with 625 mg, 1250 mg, and 2500 mg doses of the extract respectively.

Pilot Study:

On the 9th day of the experiment a preliminary clinical study was conducted on the groups (groups 3, 4 and 5) that manifested skin ulcer. Randomly selected rats were subjected to wound culture, blood culture, colony count, WBCT, WBCD and HCT level.

Microbiological Culture

After six days of antibacterial administration, groups 3 and 4 demonstrated skin ulcer. A rat with skin ulcer was randomly selected from each of the groups for wound and blood culture, and colony count. The skin surrounding the wound was sterilized with 70 % alcohol and the ulcerated surface was swab with the sterile swab stick under aseptic condition. The sample was cultured on the Manitol salt agar and incubated at 37 °C for 24 h. A blood sample was collected from the heart of the rat and subjected to blood culture and colony count. After 7 days post- administration of *P. ostreatus*, group 1 to 6 were subjected to blood culture and colony count as described by Cheesbrough, (9) with a little modification. Blood sample was collected under aseptic condition and inoculated into cooled sterile peptone water at ratio 1:10 dilution, incubated at 37 °C for 3 days. On the third day, serial dilution (10^{-1} to 10^{-5}) of one volume of cultured broth was prepared. The diluted cultured broth was then sub-cultured on freshly prepared sterile Manitol salt agar and incubated at 37 °C for 24 h. The growth was further confirmed using gram staining procedures and biochemical tests. Bacterial (*S. aureus*) colonies on the plate were counted and recorded.

Biochemical and Haematological Evaluation of Albino rats

A blood sample obtained from the rat were delivered into plain bottle and dipotasium Ethylene diamine tetraacetic acid (K_2EDTA) bottle. The serum was analyzed for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total protein, Urea and Creatinine, using enzyme maker kits from Randox. The EDTA blood sample was analysed for haematological parameters (Haematocrit (HCT) level, Leucocyte count, Leucocyte differential count (Neutrophil,

Lymphocyte, Eosinophil, Monocyte, Basophil), and Red blood cell count (RBC)) using standard operating procedures (9).

Data analysis

Data were expressed as means ± standard error of mean and subjected to one-way analysis of variance (ANOVA). Treatment means were compared using Turkey's Multiple Comparison tests with the aid of Graph Pad Prism 5 at $P<0.05$.

RESULTS

Effect of the Extract on the Temperature and weight of Albino Rats

Figure 1.1 showed numerical variation in the temperature of albino rats: Normal control showed an initial temperature of 37 °C which was maintained till the end of the experiment. All the inoculated rats had temperature variation within 36 °C to 38 °C during the incubation period and within 4 days administration of antimicrobials. Finally, after 7 days post-administration of antibacterial, control and tests maintained normal temperature except for 625 mg dose that was numerically raised.

In Figure 1.2, the average body weight during three days incubation period was reduced in all the inoculated rats; after 7 days administration of the antibacterial there was a mild increase in weight. This was followed by a drastic weight increase in group 4 after 7 days post-treatment. Normal control demonstrated gradual weight increase till the end of the experiment (90 to 96 g) while group 3 (negative control) showed a better weight increase from 87 to101 g, but a very mild increase in the positive control (90 to 92 g) (Figure 1.2). The treatments demonstrated no statistical variation in the average body weight $P<0.05$.

Clinical Changes in the Albino Rats after 6 days Administration of Antibacterial, and 7 Days Post-treatment

In the preliminary test in Table 1.1, 80 % (4 of 5) of group 3 (negative control) and 4 (625 mg) demonstrated skin ulcer (Plate 1.1) which yielded heavy bacterial growth of *S. aureus*. The negative control and 625 mg produced moderate growth of *S. aureus* in blood culture while 1250 mg yielded mild bacterial growth. After 7 days administration of antimicrobials number of colony counted in cultured blood was highest in the negative control and least in 1250 mg (Table 1.1). White blood cell total and neutrophil counts were numerically lowest in 1250 mg and highest in negative control (16.6×10^9 /L; 73 %). Consequently, lymphocyte was numerically highest in 1250 mg (30 %) and least in the negative control (27 %). Eosinophil, monocyte, and basophil were within 0 % to 1 % for the three groups. Table 1.1 showed the haematocrit level ranging from 47 % (Group 5) to 45 % (Group 3). After 7 days post-administration of the extract, colony count was significantly low in group 1 (normal control), 2 (positive control), 5 (1250 mg), and 6 (2500 mg) but high in group 3 (negative control) and 4 (625 mg) $P<0.05$ (Table 1.1)

Effect of *P. ostreatus* on Haematological and Biochemical Parameters of the Inoculated Rats

The haematological parameters of the albino rats after 7 days post-administration of *P. ostreatus* demonstrated highest White blood cell total count (WBCT) in the negative control ($14.54 \pm 2.39^b \times 10^9$/L) and least in 2500 mg dose ($6.98 \pm 1.20^a \times 10^9$/L) (Figure 1.3). Neutrophil was significantly raised in the negative control and 625 mg; lymphocyte was statistically raised in groups 1 (normal control) and 6 (2500 mg) while eosinophil, basophil and monocyte were 0.00 to 0.40 % in all the groups and were within normal range $P<0.05$ (Figure 1.3). The haematocrit level and Red cell count demonstrated no significant variation in both treatment and control $P<0.05$.

Figure 1.4 showed serum biochemical parameters of albino rats after seven days post-administration of *P. ostreatus* extract. The tests demonstrated Alanine amino transaminase (ALT) value ranging from 30.67 ± 1.76 to 49.33 ± 11.10 IU/L and within normal control (54.50 ± 7.22 IU/L) (Figure 1.4). Serum Aspartate amino transaminase (AST) was the least in the positive control (71.33 ± 20.22 IU/L) and highest in the normal control (113.00 ± 8.73 IU/L); and Alkaline phosphatase (ALP) level ranged from 34.33 ± 9.77 IU/L (negative control) to 59.67 ± 1.67 IU/L (1250 mg) (Figure 1.5). Total protein was numerically highest in 1250 mg and least in 625 mg. Figure 1.4 showed urea level ranging from 5.37 ± 0.79 Mmol/L (625 mg dose) to 8.67 ± 0.61 Mmol/L (normal control), and creatinine level was significantly raised in group 2 (positive control) 139.00 ± 2.65^c µmol/L $P<0.005$.

DISCUSSION

In the search for new antimicrobials from natural sources, mushrooms are of particular interest due to their potential as a source of secondary metabolites (10).

Therapeutic evaluation of *P. ostreatus* extract on *S. aureus* infected albino rats revealed a slightly raised rectal temperature following inoculation; which is indicative of active infection. According to Ochei and Kolhatkar (11), the rectal temperature of rats is between 36 and 40 °C. Although in all the groups, the temperature was within the normal values throughout the experiment but a gradual decline after 2 days administration of antibacterial until it dropped to the starting temperature was indicative of a positive response to treatment; consequently an improvement in the experimental animals. The only exceptional case was the slight increase in temperature after 7 days post-administration of *P. ostreatus* in 625 mg dose. This could mean incomplete elimination of the infection and might allowed proliferation after a full course treatment. It could be inferred that 625 mg/kg body weight was not adequate to eradicate *S. aureus* infection.

The initial reduction in the average body weight observed within the first 3 days of the experiment could be attributed to the effect of the bacterial on the body system and the process of the immune system in response to the foreign substance. The mild weight increase in *P. ostreatus* treatment as compared with better weight gain in the negative control could indicate that bioactive compounds in *P. ostreatus* did not encourage weight gain and might be of value in limiting obesity and maintaining normal body weight in health (12). The mild weight gain obtained in the experimental animals treated with extract is similar to the work of some researchers (13, 14, 15) who reported the benefits of *Pleurotus* spp. in reducing inflammation and weight gain. It also corroborates the study of Fard *et al*. (16) who reported lower body weight in chickens fed with 2 % mushroom supplement.

Skin ulcers observed in the negative control and 625 mg dose could result from *S. aureus* infection. *P. ostreatus* administered led to significantly reduced colony count; indicating its potency against the pathogens; and appreciable improvement was obtained with an increased dose. The healing effect observed in the experimental animals was dose-dependent.

Pleurotus ostreatus dosage ranging from 1250 to 2500 mg/kg body weight was effective against pathogenic *staphylococcus*. The average colony count in seven days post-treatment cultured blood also indicated that *P. ostreatus* administration at 1250 to 2500 mg doses produced insignificant low colony count that was comparable with 13.33 mg Ciprofloxacin and placebo while 625 mg could not produce recovery. It could be inferred that *P. ostreatus* at 1250 to 2500 mg dose compete favorably well with 13.33 mg Ciprofloxacin; and able to maintain the clinical recovery without relapses. However an under-dose treatment may not be effective against killing or eradication of the pathogenic bacteria (17). *P. ostreatus* may contain bioactive compounds that are potent *in vivo* against *S. aureus* infection.

Blood leucocytes are body soldiers that are involved in eliciting an immune response against infectious agents and are raised in bacterial infection. In the study, leukocytes and neutrophils were reduced gradually with increased dose. Significant leukocytosis due to neutrophilia was observed in the negative control and 625 mg treatment. The colony count and haematological results were in harmony and discouraged the administration of 625 mg/kg *P. ostreatus* against *Staphylococcus aureus* infection. Lymphocytes are moderately lower in the system when compared with neutrophils in health; they are increased at a young age and reduced in viral trauma, impaired lymphopoiesis (immunosuppression), and immunodeficiency; in this study, the lymphocytes were within normal control range. Eosinophils are raised in some parasitic infection while increased monocytes and basophils values are not clinically significant but in this study, they were maintained within the normal range. The test rats demonstrated haematocrit levels that was comparable to normal control and indicative of normal erythropoiesis. This could be attributed to the nutritional values derived from the extract.

A seven days' administration of *P. ostreatus* was beneficial to the serum enzymes and kidney markers. Alanine amino transaminase (ALT), Aspartate amino transaminase (AST) and Alkaline phosphatase (ALP) are biomarkers of liver function test; they are raised in metabolic disorders with secondary liver disease. The ALT, AST, and ALP were normal in all the treatments indicating that *P. ostreatus* had no deleterious observable effect on the liver enzymes which may indicate no hepatocellular damage. A decrease in serum protein is an indication of impaired synthetic functions of the liver. The protein level was within the normal range indicating that *P. ostreatus* posed no danger to the hepatocytes and liver enzymes. Serum urea and creatinine are reliable markers of renal functions and the extent of renal damage is usually assessed by increased serum level of these enzymes, however, this study demonstrated a decrease

compared to normal serum urea and creatinine level throughout the graded doses. This suggests that *P. ostreatus* at doses up to 2500 mg/kg might not be harmful to renal cells.

CONCLUSION

P. ostreatus at doses ranging from 1250 to 2500 mg are potent against *S. aureus* infection whereas a low dose of 625 may not produce a cure against pathogenic *S. aureus.* The biochemical properties are suggestive of safety and tolerance of the bioactive agents present in the mushroom to the body system. This information could be useful in the production of new antimicrobials of commercial value which will be less toxic to the body system. However further studies are required to isolate the bioactive compounds inherent in the mushroom species.

Conflict of Interest: There is no conflict of interest.

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Table 1.1: Clinical Changes in the Albino Rats after 6 days Administration of antibacterial and 7 days Post-treatment

G	Skin ulcer (%)	W/S culture	Blood culture	Cc 6 ($\times 10^3$ Cfu/ml)	Cc 7Post ($\times 10^4$ Cfu/ml)	WBCT (10^9 /l)	N (%)	L (%)	E (%)	M (%)	B (%)	HCT (%)
G1	0	NA	NG	NA	2.00 ± 0.63^{a}	NA	NA	NA	NA	NA	NA	NA
G2	0	NA	NG	NA	2.00 ± 1.00^{a}	NA	NA	NA	NA	NA	NA	NA
G3	80	+++	++	110	32.00 ± 6.10^{b}	16.6	73	27	00	01	00	45
G4	80	+++	++	95	43.40 ± 6.20^{b}	15.2	71	28	00	01	00	47
G5	0	NA	+	36	5.60 ± 3.20^{a}	13.4	69	30	00	01	00	47
G6	0	NA	NA	NA	1.80 ± 1.10^{a}	NA	NA	NA	NA	NA	NA	NA

key: g1= normal control; g2 = positive control; g3 = negative control; g4 = 625 mg dose; g5 = 1250 mg dose; g6 = 2500 mg dose; wbct = total white blood cell count; n = neutrophil; l = lymphocyte; e = eosinophil; m = monocyte; b = basophil; g = group; na = not applicable; ng = no growth; cc 6 = colony count after 6 days administration of antibacterial; cc 7post = colony count after 7 days post- administration of antibacterial, different superscripts along the same vertical axis at P< 0.05 is significant

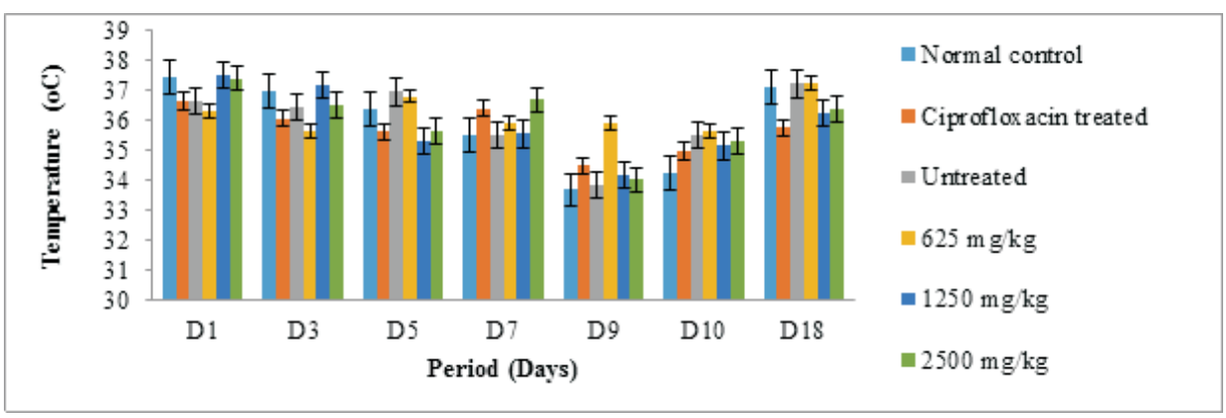

Figure 1: Effect of *P. ostreatus* on the temperature of albino rats inoculated with *S. aureus*

key: d1= day 1 of the experiment; d3 = day 3; d5 = day 5; d7= day 7; d9 = day 9; d10 = day 10; d18 = day 18; untreated = negative control;

***p < 0.05 is a significant difference**

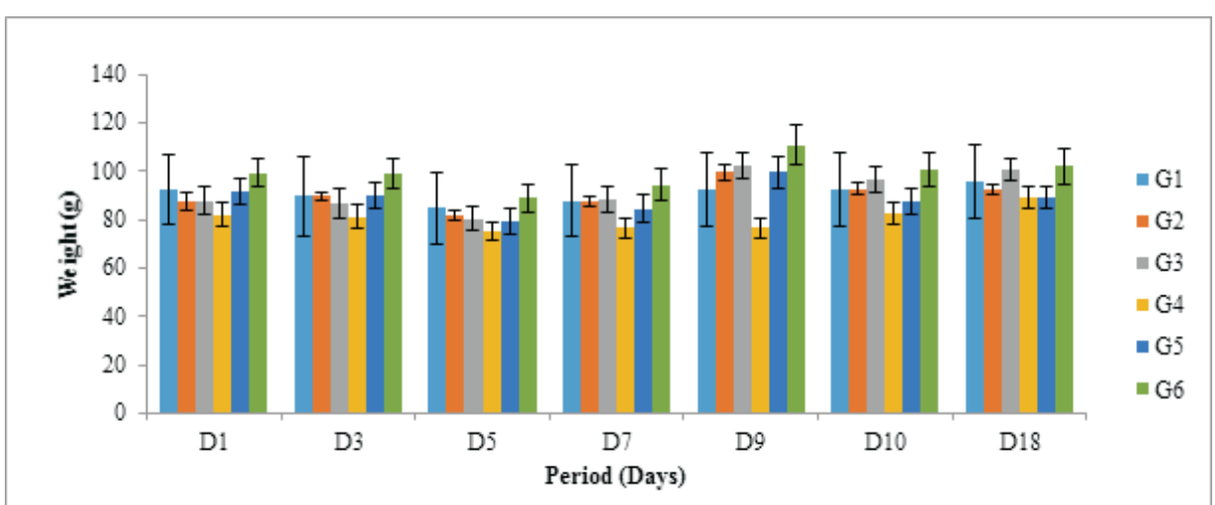

Figure 2: Effect of *P. ostreatus* on the weight of albino rats inoculated with *S. aureus*

key: d = day; g1 = normal control; g2 = ciprofloxacin was treated (positive control) ; g3 = negative control; g4= 625 mg dose; g5 = 1250 mg dose; 2500 mg dose;

***p < 0.05 is a significant difference**

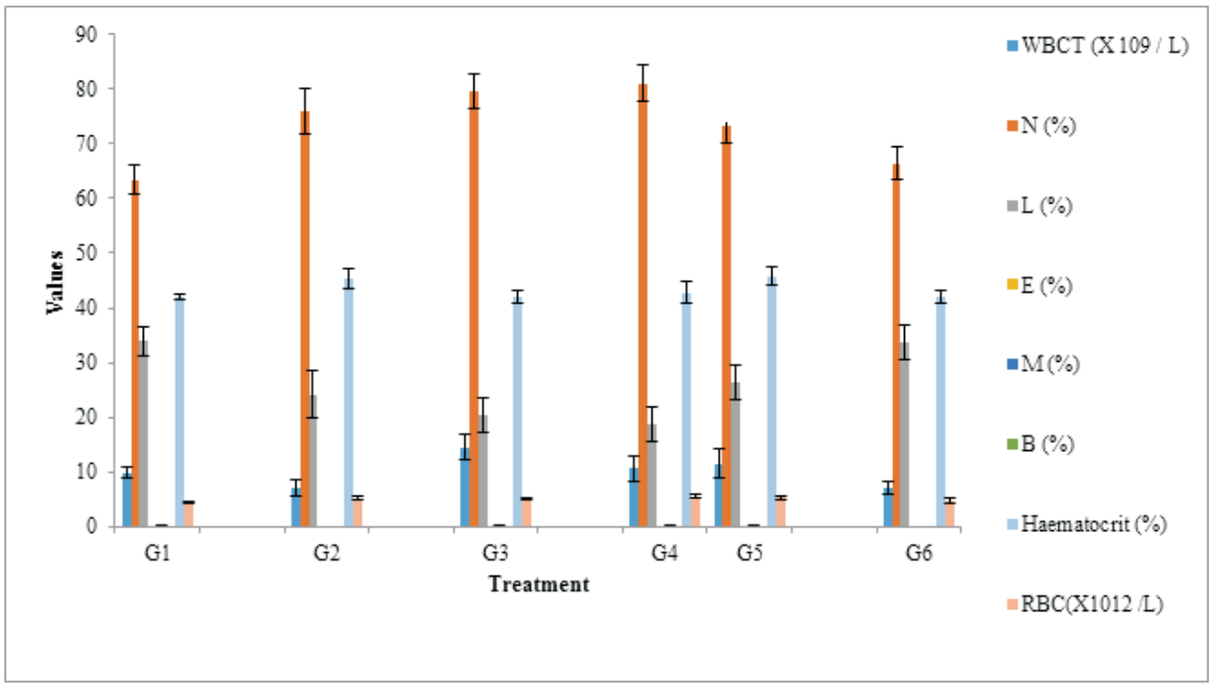

Figure 3: Effect of *P. ostreatus* on haematological parameters of albino rats inoculated with *S. aureus*

key: wbct = total white blood cell count; rbc = red cell count; n = neutrophil; l = lymphocyte; e = eosinophil; m = monocyte; b = basophil; n = 5; g1 = normal control; g2 = ciprofloxacin treated; g3 = negative control; g4 = 625 mg; g5 = 1250 mg and g6 = 2500 mg *($p < 0.05$) is a significant difference.

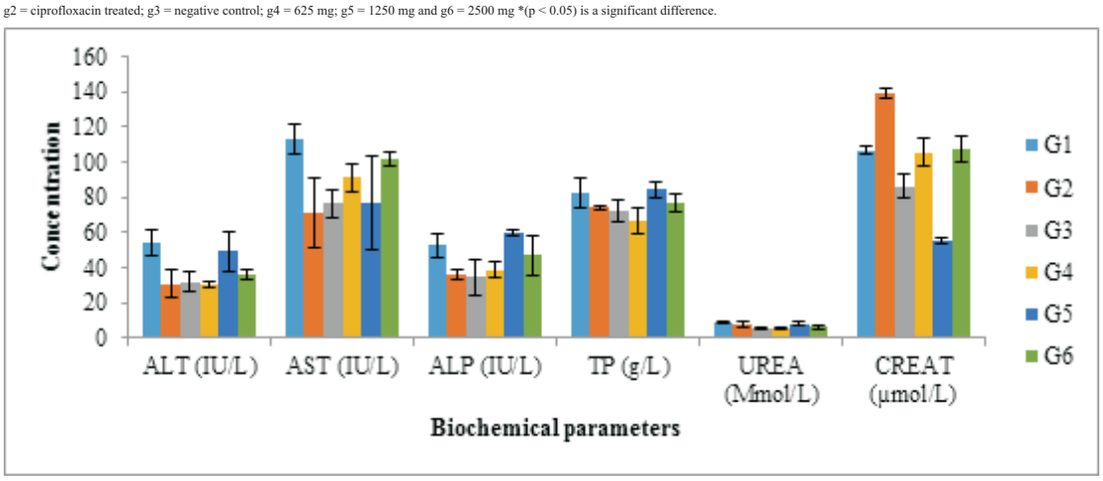

Figure 4: Effect of *P. ostreatus* on biochemical parameters of albino rats inoculated with *S. aureus*

key: alt = alanine amino transaminase; ast = aspartate amino transaminase; alp = alkaline phosphatase; tp = total protein; creat = creatinine; g1 = normal control; g2 = ciprofloxacin treated; g3 = negative control; g4 = 625 mg dose; g5 = 1250 mg dose and g6 = 2500 mg dose *($p < 0.05$) is a significant difference

A

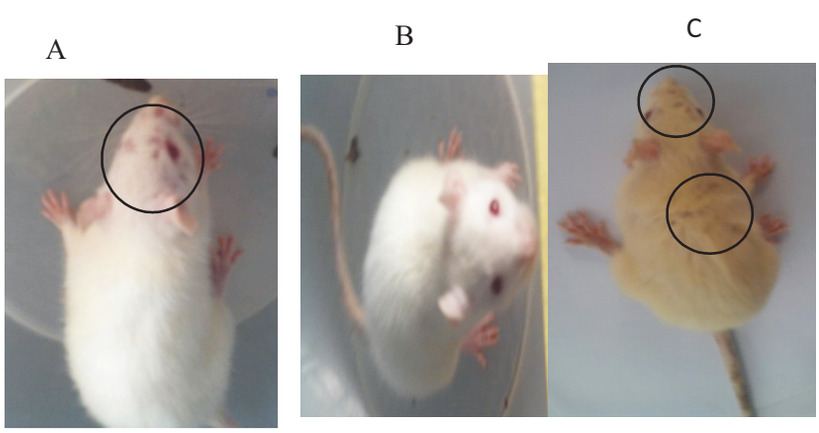

Plate 1.1: Wistar albino rats in *S. aureus* infection showed ulcerated skin (ring) in 625 mg (A) and the negative control (C) as compared with healthy skin in normal control (B) × 0. 05