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Original Article

Immunological Response of Albino Rats Immunized With Heat-Killed Candida Albicans for the Possible Prevention of Candidemia

Ahmad Ibrahim[*1], Yahaya Abdullahi Umar[1] and Ahmed Babangida Suleiman[2]

[1]Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria
[2] Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria

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Corresponding Author
Ahmad Ibrahim
ahmadibrahim337@yahoo.com

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ABSTRACT

The treatments of systemic candidiasis in humans with the current antifungal drugs of choice such as azole derivatives and amphotericin B have raised issues associated with toxicity, resistance, high morbidity, socioeconomic impact, and low quality of life. The immunogenic property of heat-killed *C. albicans* to elicit immunological response in the possible prevention of the candidiasis was explored. At baseline, white blood cell differentials and antibody titers were determined after acclimatization. The experimental group was immunized on two occasions with 10^6cells/ml of heat-killed *C. albicans* and subsequently challenged along with the positive control with 10^6 viable *C. albicans* while the Negative control received normal saline during the same period of the study. These rats were bled for the determination of antibody titers and white blood cell differentials. Also, rats from the three groups were observed for four weeks for survival after challenge to determine the protective effect of heat-killed C. albicans. Delayed- type hypersensitivity of rats' footpads were also checked to determine the role of cell- mediated immunity The results revealed that heat killed *C. albicans* stimulated a significant amount of antibodies and WBC differentials that were immunoprotective. We also found that all immunized rats survived challenge with 10^6 viable cells while the kidneys of the dead unimmunized rats showed a positive growth of *C. albicans*. Thus, heat killed *C. albicans* could provide significant protection.

Keywords: Candida albicans, Candidaisis, Delayed-type hypersensitivity, Prevention.

INTRODUCTION

Candida albicans is the most common candida specie that causes candidiasis or yeast infection (1, 2). It is reported to be present in 80% of human population as commensal but also has the ability of transiting to pathogenic forms when there is an imbalance of the normal microbiological flora, breakage of epithelial barriers or dysfunction of the immune system (3, 4). These conditions may result from a wide-broad spectrum use of antibacterial, surgery, mutation or heredity among other factors (5). Bloodstream infection or systemic infection is initiated when *Candida albican*s gain access to the blood stream and invade the organs (6) and hence associated with kidney or brain damage (7). In addition, candidemia a life threatening infection accounts for 50-70% of all cases of invasive mycoses (9). Despite the public health significance of candidiasis associated with of toxicity, resistance, high morbidity, socioeconomic impact, low quality of life or even death, past studies in Nigeria on development of prophylactic options for the prevention of systemic candidiasis is scanty. Moreover, treatment of candidiasis depends on the use of chemotherapy which is usually toxic, costly and most a times not available. More disturbing is the emergence of resistance to most of the commonly used antifungal drugs (10). These limitations have raised the need for alternative source of treatment or prevention that is effective, and safe with no side effects (10).Therefore, the objective of this study therefore is to explore the potential of heat killed C. albicans sensitized host immune cells for the prevention of *C. albicans* infection.

MATERIALS AND METHODS

Experimental Animals

Male albino rats of 6-7 weeks old were used in this study. The rats were provided with water and food *ad libitum* and allowed to acclimatize for 7 days prior to the commencement of the experiment. The use of animal for this study was approved by the Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Research and Development on the standard practices of handling experimental animals in line with international best practice.

Vaccine Preparation

Candida albicans isolate was obtained from a clinical sample of a patient suspected with *candida vaginitis.* An informed consent was sought from the patient before the collection of the sample. The sample was cultured on sabouraud dextrose agar at 37^{o}C for 2 hours (10). The creamy, white and pasty appearance of *C. albicans* produced on SDA was sub-cultured on a chromogenic agar (colour based selective medium) at 37^{o}C for 48 hours (11). This was followed by a germ- tube test to differentiate albicans from non- albicans. Germ tube test was achieved by inoculation of a portion of *C. albicans* in serum and incubated at 37^{o}C 3 hours (12). This preparation was viewed under the microscope for the presence of filament or outgrowth. *Candida albicans* used for immunization was suspended in a sterile normal saline and heated in a water bath at 65^{o}C for 6 hours. Non-viability was confirmed by culturing on SDA for 24 hour at 37^{o}C (13).

Experimental Design

Three types of experiments were carried out namely: (1)determination of variation of antibody titres, white blood cell differentialsand CD4 (2) delayed type hypersensitivity and (3)survival analysis and determining Candida albicans growth in kidneys of dead rats.

Immunization and Challenge

The first experiment was performed to determine variation or abnormalities of antibody titres, white blood cells differentials and CD4. Hence, rats were divided into fifteen rats each of three groups namely; negative control, positive control and immunized group. Baseline was taken for all the rats 7 days after acclimatization. The immunized group received subcutaneous injection of 1×10^6 heat killed *C. albicans* while the negative and positive controls received same concentration and route of viable cells and sterile normal saline respectively. After 21 days, the rats received a booster injection of same preparation and through the same route. This is followed by an intravenous (caudal) challenge with viable *C. albicans* after 14 days of booster injection. The rats were finally sacrificed and their blood collected by cardiac puncture after 3 days of challenge. The following parameters were determined using relevant assays as detailed below to check abnormalities.

Determination of WBC Differentials

Blood samples were collected in EDTA bottles and analyzed with the aid of an automated hematology analyzer (Abacus 380) to determine the frequency of abnormalities.

Determination of Serum Antibody

This was achieved as described by Bin-Hafeez *et al.*, (14). Blood samples were collected in plane bottles and centrifuged at 2500 rpm for 10 minutes and sera obtained using a micropippete. As much as 100µl of serum was heat-inactivated at 56^{o}C in water bath for 30 minutes. About 50µl of phosphate buffered saline (PBS) was then added to all 12 tubes. The first tube was considered the control hence it only received 50µl of PBS, while the second well received 50µl of heat-inactivated serum to form a mixture of serum and PBS. From the second tube, 50µl of the mixture was used to serially dilute by 2-fold in the subsequent tubes. Finally, 50 µl of heat killed *C. albicans* with a cell density of 10^6 cells/ml was added to all the tubes and incubated at 37^{o}C for 2 hours. The values of antibody titre were assigned to the highest serum dilution showing at least 50% of visible agglutination.

Delayed-Type Hypersensitivity

The second experiment was performed according to Evron *et al.*, (13). The rats were immunized as in the first experiment except that they were not challenged and sacrificed. Two groups of five rats each were used as control and immunization groups. After 21 days of sensitization, there footpads were injected with 0.01 ml of normal saline containing 10^6 inactivated *C. albicans* and sterile normal saline to the immunized and control groups respectively. Their footpads were measured with a Vernier Caliper before injection (at 0 hour). Footpads' thicknesses of challenged rats were then measured at 24 and 48 hours after challenge. The mean values obtained were compared.

Determination of Daily Survival Analysis

The third experiment was carried out to determine survival of the rats after challenge. This was achieved according to Thomas *et al.*, (15) method. They

were immunid and challenged as described in the first experiment except that the rats were not sacrificed but monitored for a period of 28 days after challenge. Kidneys of dead rats during this period were harvested. The kidneys were sectioned, stained with crystal violet stain (microscopic stain BDH chemicals Ltd) and viewed under X 500 digital microscope (Coolinttech, UK). Also homogenized portion were cultured on agar plate containing chloramphenicol. This is to check for *C. albicans* growth.

Compliance with Ethical Standards

There is no conflict of interest. The use of rats in this study is in accordance with the international best practices approved by the department of Pharmacology and Toxicology, National Institute of Pharmaceutical Research and Development, Abuja. The patient was also consented before the sample was taken for identification

Statistical Analysis

The data collected from this study were subjected to statistical analysis. Analysis of variance (ANOVA), student t-test and simple percentage were used to analyze these parameters. Analyses were performed using VassarStat Software (USA).

RESULTS

Antibody Titers of Rats Immunized with Heat killed *Candida albicans*

Result of antibody titers of rats showed that vaccination with inactivated *C. albicans* stimulated significant ($P<0.05$) amount of antibodies compared to controls (Figure 1). The mean baseline antibody titer of the rats taken after 7 days of acclimatization was 25.6 ±10.55 for all the groups. After 38 days of vaccination, booster injection and challenge, mean antibody titers recorded for the negative control group increase slightly to 32 ± 8.76 from the baseline titers of 25.6 ±10.55. The difference between antibody titers of rats in the negative control when compared to the baseline titers did not show any statistically significant difference ($P> 0.05$).

However, in the Heat treated group, there was a statistically significant ($P<0.05$) increase (1228.8 ± 347.2) in antibody titers when compared with control groups after 38 days of intermittent immunizations and challenge with inactivated *C. albicans* and viable cells respectively. The positive control which was not immunized but infected during the same period of the study showed a statistically significant ($P<0.05$) lower mean antibody titers (256 ± 70.11) when compared to the immunized group (Table 1).

White Blood Cell of Albino Rats Immunized with Heat killed *Candida albicans* and Controls

The WBC count of albino rats vaccinated and challenged with *C. albicans* is presented in Figure 2. The mean white blood cell counts (WBC) after 7 days of acclimatization (baseline before immunization and subsequent challenge) of the rats was 7.6 ± 0.3195. After 38 days, the mean white blood cell counts of rats in the negative control group increased slightly to 8.8 ± 0.3458. In addition, subsequent immunizations with inactivated *C. albicans* and challenge with viable yeasts resulted in a statistically significant ($p<0.05$) increase in mean white blood cell counts compared to both baseline counts and the positive control group. Rats in the immunized group recorded relatively higher mean WBC counts of 10.58 ±0.7702 f. However, rats in the positive control

group had decreased WBC counts of 5.12 ± 0.3385 which is the lowest when compared to all groups. Statistical analysis shows a significant difference (P<0.05) in WBC counts of rats in the immunized and positive control groups (Table 2).

Table 1: Antibody Titers of Rats Vaccinated with Inactivated *Candida albicans* and Controls

Vaccination status	Mean Antibody Titres (µg/ml)		Level of significance	
	Day 7 (Baseline)	Day 38	(P<0.05)	(P>0.05)
Heat inactivated *C. albicans*	25.6 ±10.55	1228.8 ± 347.26	H vs B	
Negative Control	25.6 ±10.55	32 ± 8.76		U vs P
Positive Control	25.6 ±10.55	256 ±70.11	H vs P	

Keys: **N: Negative control**, **P: Positive control**, **H: Heat inactivated *C. albicans***, **B: Baseline**

Table 2: White blood cell count of rats vaccinated with inactivated *Candida albicans* and controls

Vaccination status	Mean WBC counts (%)		Level of significance	
	Day 7 (Baseline)	Day 38	(P<0.05)	(P>0.05)
Heat inactivated *C. albicans*	7.6 ± 0.3195	10.58 ± 0.7702	H vs B	
Negative Control	7.6 ± 0.3195	8.8 ± 0.3458		U vs P
Positive Control	7.6 ± 0.3195	5.12 ± 0.3385	H vs P	

Keys: **N: Negative control**, **P: Positive control**, **H: Heat inactivated *C. albicans***, **B: Baseline**

Table 3: Mean Percentage Lymphocyte Counts of Rats Vaccinated with Inactivated *Candida albicans* and Controls

Vaccination status	Mean Lymphocyte counts (%)		Level of significance	
	Day 7 (Baseline)	Day 38	(P<0.05)	(P>0.05)
Heat inactivated *C. albicans*	67.42 ± 1.3291	74.98 ± 1.4824	H vs B	
Negative Control	67.42 ± 1.3291	66.32 ± 1.1517		U vs P
Positive Control	67.42 ± 1.3291	57.06 ± 1.6382	H vs P	

Keys: **N: Negative control**, **P: Positive control**, **H: Heat inactivated *C. albicans***, **B: Baseline**

Table 4: Mean Percentage Granulocytes of Rats Vaccinated with Inactivated *Candida albicans* and Controls

Vaccination status	Mean Granulocyte counts (%)		Level of significance	
	Day 7 (Baseline)	Day 38	(P<0.05)	(P>0.05)
Heat inactivated *C. albicans*	25.56 ± 0.8298	21 ± 0.9752	H vs B	
Negative Control	25.56 ± 0.8298	25.94 ± 1.061		U vs P
Positive Control	25.56 ± 0.8298	15.7 ± 0.584	H vs P	

Keys: **N: Negative control**, **P: Positive control**, **H: Heat inactivated *C. albicans***, **B: Baseline**

Table 5: Mean Percentage MED (monocyte, eosinophils and basophils) of Rats Vaccinated with Inactivated *Candida albicans* and Controls

Vaccination status	Mean MEB counts (%)		Level of significance	
	Day 7 (Baseline)	Day 38	(P<0.05)	(P>0.05)
Heat inactivated *C. albicans*	3.66 ± 0.16	5.26 ± 0.5381	H vs B	
Negative Control	3.66 ± 0.16	4.32 ± 0.4247		U vs P
Positive Control	3.66 ± 0.16	27.28 ± 1.3869	H vs P	

Keys: **N: Negative control**, **P: Positive control**, **H: Heat inactivated *C. albicans***, **B: Baseline**

Table 6: CD4 Counts of Immunized and Control Groups

Rat groups	Mean ± SE	Level of Significance
Heat group	14 ± 0.71	
Negative Control	9.6 ± 0.40	P < 0.05
Positive Control	6 ± 0.45	

Table 7a: Delayed – Type Hypersensitivity of Control Albino Rats at 0, 24 and 48 hours

ANOVA- Standard weighted-means analysis

Source	SS	df	MS	F	P
Treatment [between groups]	0.00144	2	0.00072		
Error	0.0076	12	0.000633	1.14	0.352142
Total	0.00904	14			

Table 7b: Delayed – Type Hypersensitivity of Immunized Albino Rats at 0, 24 and 48 hours

ANOVA- Standard weighted-means analysis

Source	SS	df	MS	F	P
Treatment [between groups]	0.032573	2	0.016287		
Error	0.0092	12	0.000767	21.24	0.000114
Total	0.041773	14			

Table 8: Percentage mortality rate of rats immunized with Heat inactivated and Control groups

Vaccination Status	% Survival			
	Week 1	Week 2	Week 3	Week 4
Heat group	0.0	0.0	0.0	0.0
Negative Control	0.0	0.0	0.0	0.0
Positive Control	75.0	25.0	0.0	0.0

Lymphocytes Profile of Albino Rats Vaccinated with Heat Inactivated *C. albicans*

Albino rats in all the four groups recorded an increase in mean lymphocyte counts after 38 days as follows: Heat inactivated (74.98%), Negative control (66.32%) and Positive control (57.06%) (Figure 3). Although the increase in lymphocyte counts relative to the baseline counts (67.42%) in all the groups was not significant (P>0.05), the mean lymphocyte counts of rats in the vaccinated groups shows a

significant difference (P<0.05) when compared to rats in the control group after 38 days. Similarly, there was a significant difference in lymphocyte counts after 38 days when negative and positive control groups were compared (P<0.05). However, mean lymphocyte counts of rats in positive control group at day 38 (57.06%) shows a significant decrease when compared with lymphocyte counts at day 7 (baseline) (67.42%).

Granulocytes Profile of Rats Vaccinated with Inactivated *C. albicans* and Control

The mean percentage granulocytes of immunized and control groups is presented in Figure 4. The mean percentage granulocyte counts of rats after acclimatization (baseline) in all the groups was 25.94 ±1.061. At day 38, rats in the negative control group recorded an insignificantly slight increase (25.94 ± 1.061) in the level of granulocytes (P>0.05). However, albino rats in Heat inactivated and positive control groups recorded significantly lower (P>0.05) granulocyte values of 21% and 15.7% as compared to the initial (baseline) granulocyte values of 25.56% at day 7 (Table 4).

Mid- Range Cells (MEB) Profile of Rats Vaccinated with killed *C. albicans* and Control

The result of percentage Mid-range cells (monocytes, eosinophils and basophols) for immunized and control albino rats after acclimatization and subsequent treatment is presented in Figure 5. The mean percentage of mid-range cells for rats in all groups after 7 days of acclimatization (baseline) was 3.66± 0.16 with the exception of rats in the positive control group that recorded a significantly high MEB at day 38, rats in Heat inactivated and negative control groups recorded MEB ranging between 4.32 and 5.82%. Although the increase in MEB at day 38 in the two groups was not significantly different from the MEB recorded at day 7, and between the two treatment groups at day 38, there was a significant difference in MEB recorded when compared to positive control groups (P<0.05) (Table 5).

CD4 Counts of Albino Rats Vaccinated with Inactivated *Candida albicans* and Controls

Figure 6 shows the CD4 counts of rats in Heat inactivated, positive and negative control groups. At day 38, rats in the negative control group recorded a mean CD4 counts of 9.6 ± 0.4 compared to rats in the immunized group and positive control group with mean CD4 counts of 14 ± 0.7071 and 6 ± 0.4472 respectively. Statistical analysis shows a significant difference in CD4 counts between rats in the three groups (P<0.05) (Table 6).

Delayed-Type Hypersensitivity

The effect of sterile normal saline (control) and inactivated *C. albicans* on the footpads of treated albino rats to determine delayed type hypersensitivity is shown in Figure 7. Albino rats administered normal saline showed no significant increase in pad size at 0 hour, 24 hours and 48 hours post administration. However, albino rats immunized with inactivated *C. albicans* exhibited delayed- type hypersensitivity with mean footpad size of 0.394mm ± 0.008 at 0 hour, 0.48mm ± 0.012 at 24 hours and 0.502mm ±0.016 at 48 hours. Although the increase in mean footpad size of rats immunized with inactivated *C. albicans* was not significant (P>0.05) at 24 and 48 hours, a statistically significant (P<0.05) difference in footpad size was

observed when compared with mean footpad size of rats at 0 hours. Also, when footpad sizes of the control and immunized groups were compared at 0 hour, no statistically significant difference (P>0.05) was observe (0.396 ±0.011 and of the immunized group 0.394 ±0.0081). However, 24 hours after, footpad size of rats in the control and the immunized group increased to 0.42 ± 0.029 and 0.48 ± 0.027 respectively. Similarly, there was an increase in footpad sizes of rats in immunized group and a slight decrease in the control group after 48 hours. However, the increase in footpad size in control and immunized group showed no significant (P>0.05) difference of rats immunized with inactivated *C. albicans* and control (Table 7).

The result of survival analysis of rats immunized with Heat inactivated *C. albicans* and control groups after 28 days is presented in Figure 8. No death was recorded in rats from negative control and Heat inactivated groups during the four weeks of observation. However, 75 and 25 percent of rats in the positive control group died in the first and second weeks respectively (Table 8).

Kidney Fungal Burden

The fungal burden in kidney of dead rats is presented in Plate I, as black spots in the sectioned and stained kidneys of rats. In addition, fungal (*C. albicans*) growth was observed when homogenized kidneys of dead rats were cultured on SDA plates (Plate II).

Survival Analysis

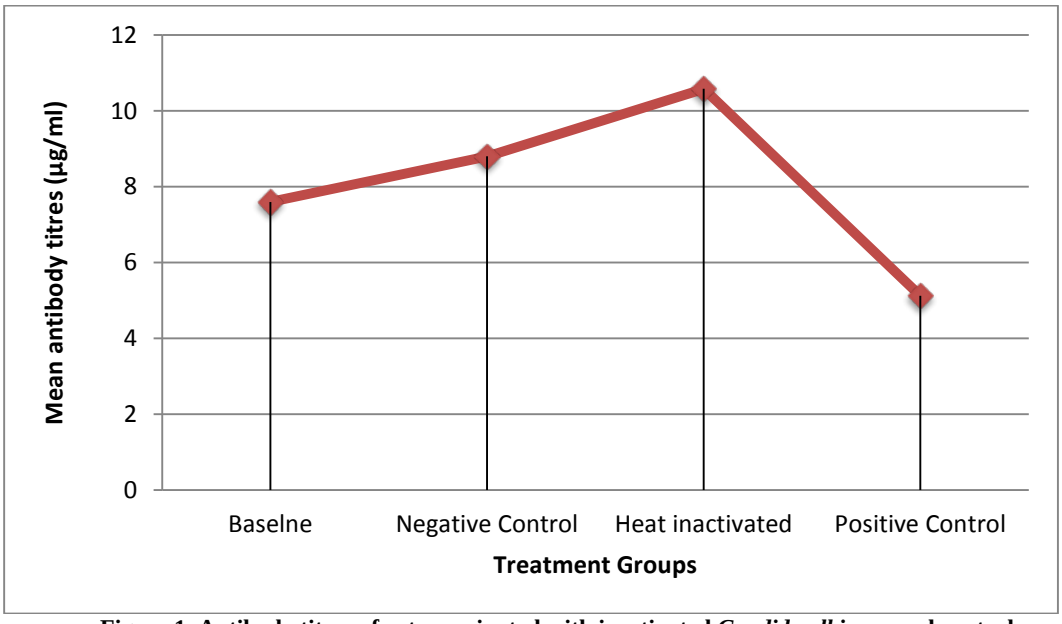

Figure 1. Antibody titres of rats vaccinated with inactivated *Candida albicans* and controls

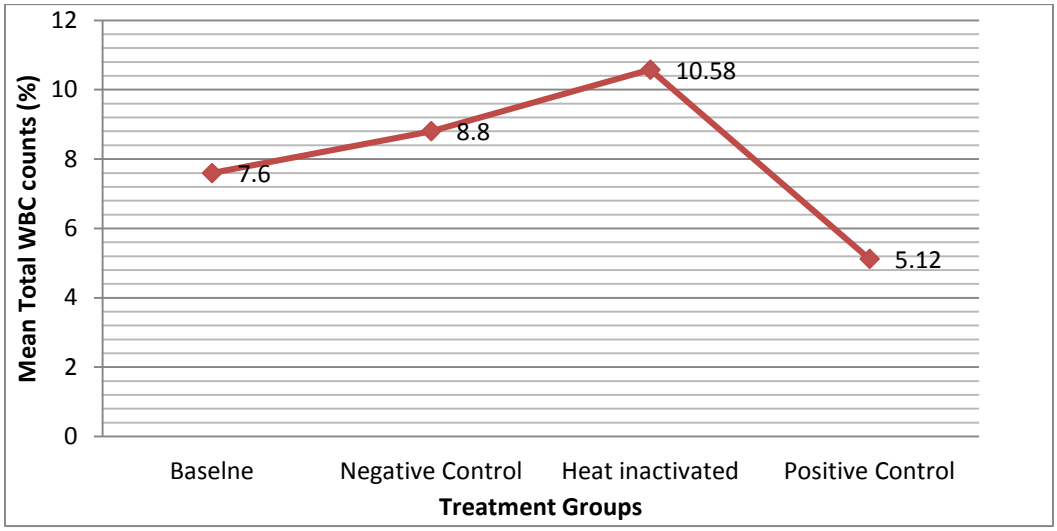

Figure 2: White blood cell count of rats vaccinated with inactivated *Candida albicans* and controls

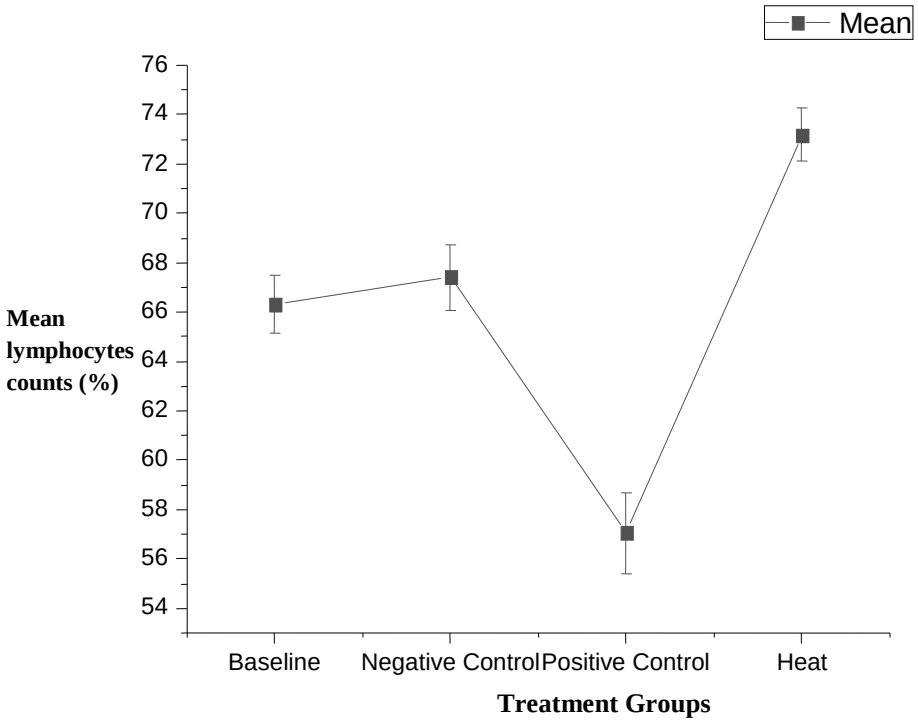

Figure 3. Lymphocytes Profile of Albino Rats Vaccinated with Heat Inactivated *C. albicans*

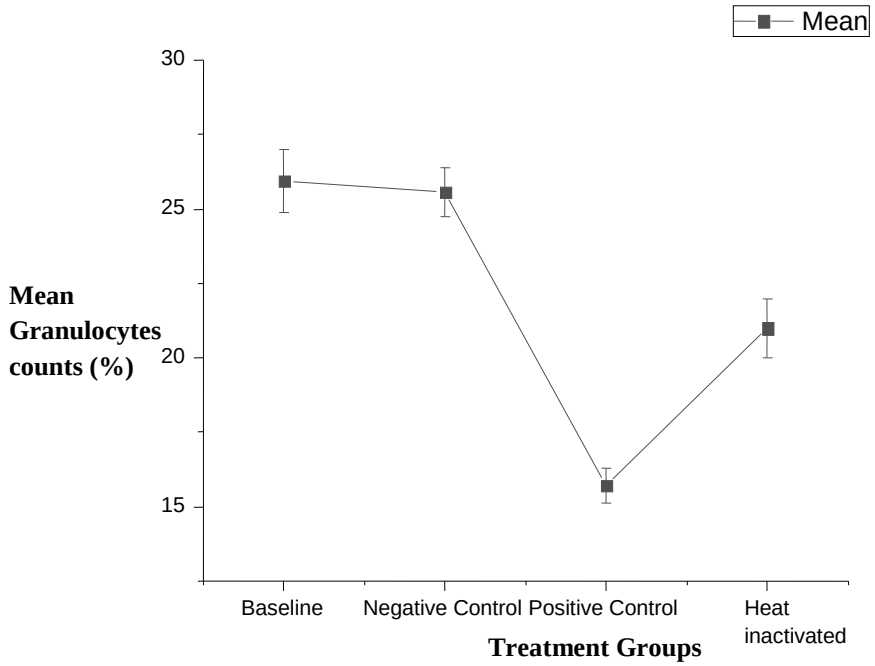

Figure 4: Granulocytes Profile of Rats Vaccinated with Inactivated *C. albicans* and Control

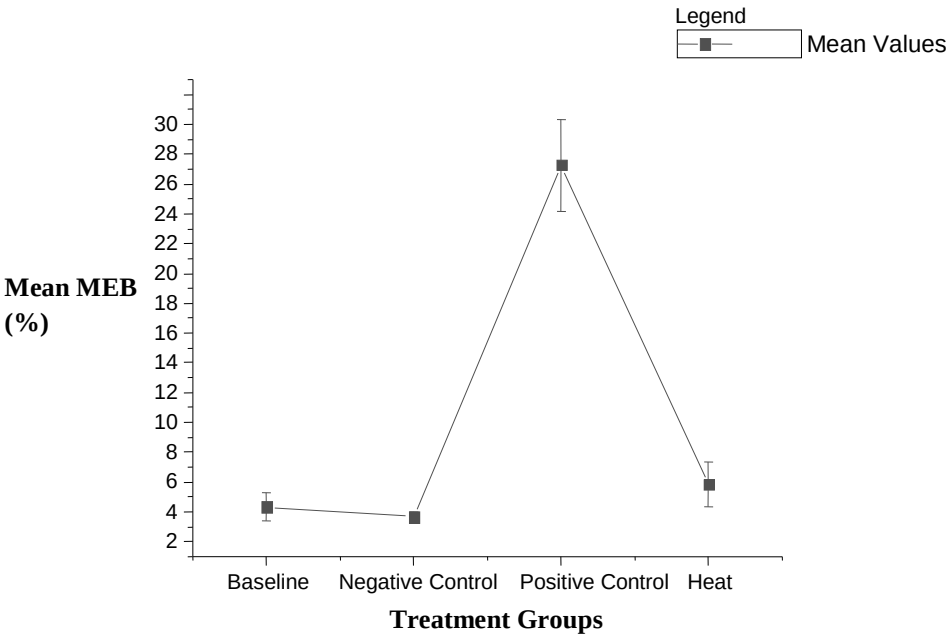

Figure 5: Mid - Range Cells Profile of Rats Vaccinated with Inactivated *C. albicans* and Control

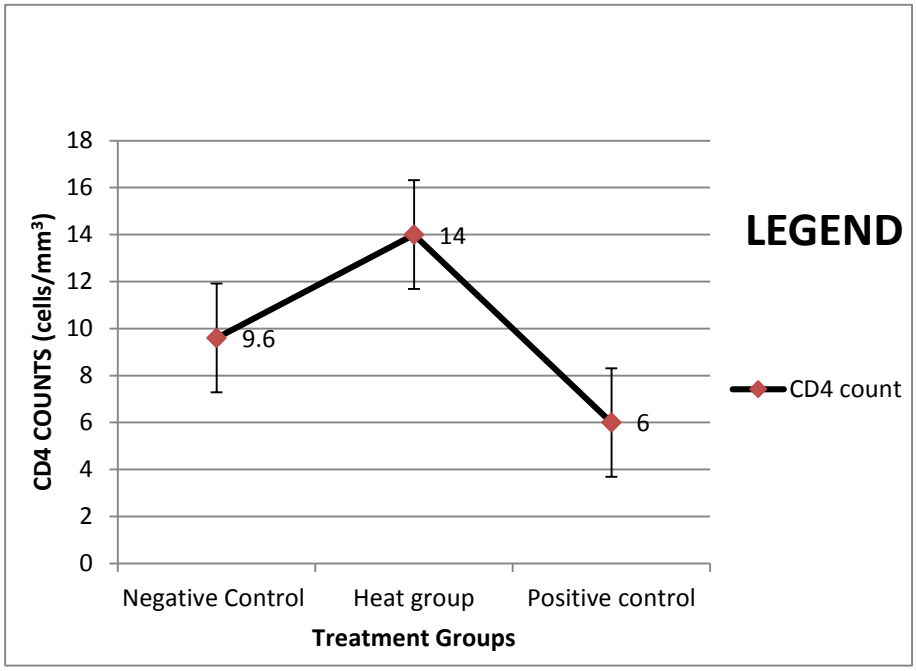

Figure 6: CD4 Counts of Albino Rats Vaccinated with Inactivated *Candida albicans* and Controls

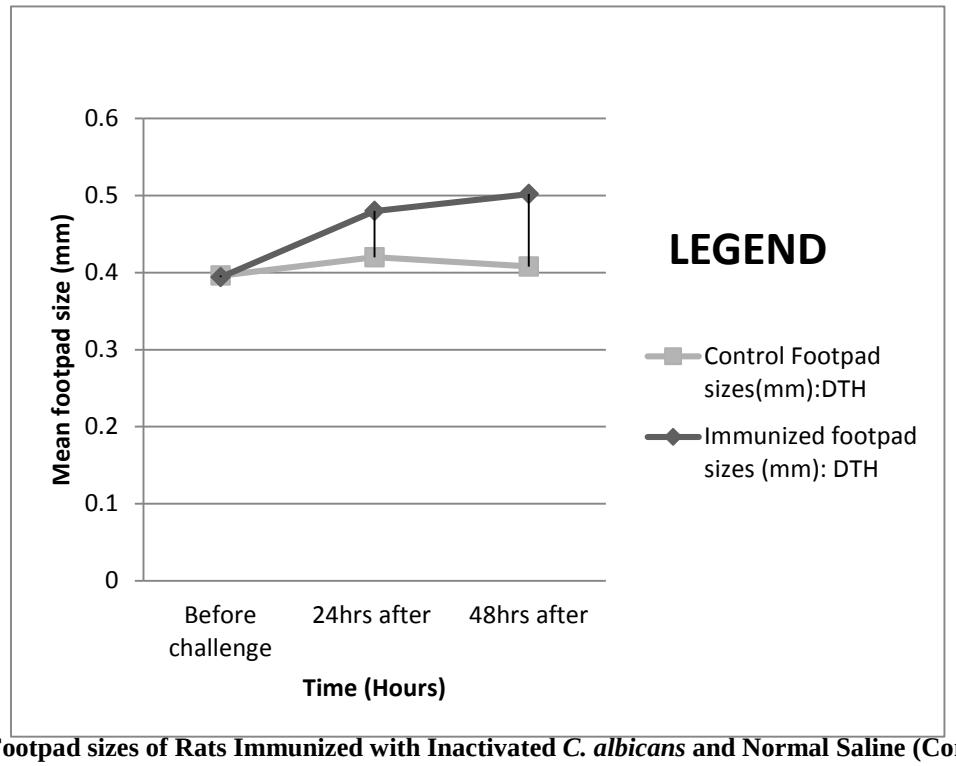

Figure 7: Footpad sizes of Rats Immunized with Inactivated *C. albicans* and Normal Saline (Control)

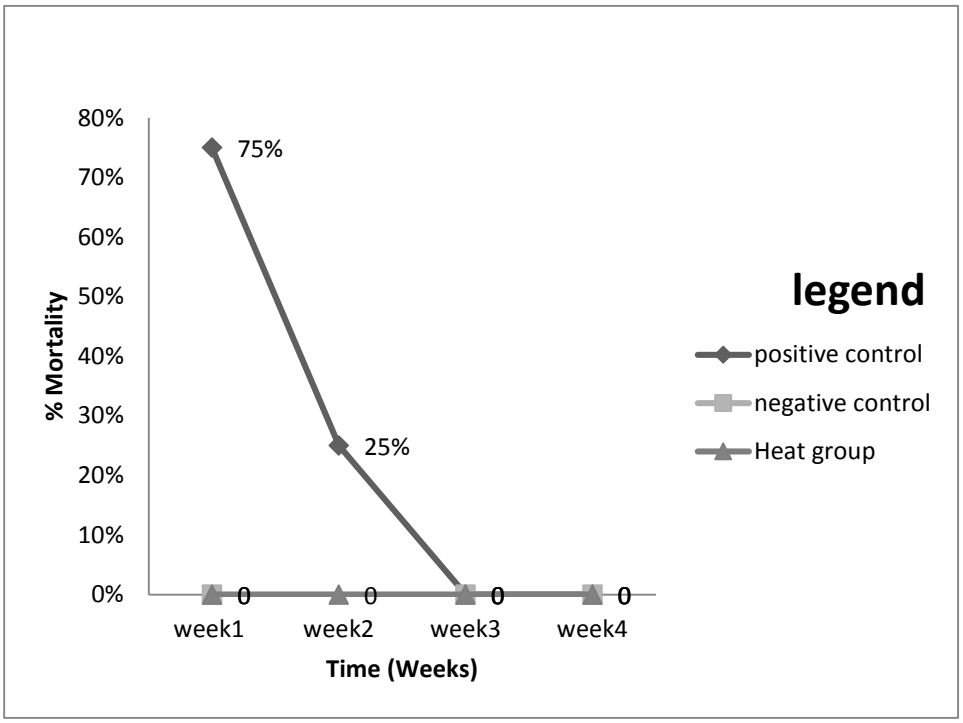

Figure 8: Percentage mortality of rats immunized with Heat and UV inactivated *C. albicans* and Control groups.

Plate I: **Sectioned and stained kidney of fungal infected albino rat (X500 magnification)**

Plate II: Fungal growth of homogenized and cultured kidney

DISCUSSION

It is well known that vaccines are developed for the prevention of diseases. They are derived from cell surface molecules, engineered organisms that have lost their virulence and pathogenecity or even killed organism that are immunogenic or have the ability of stimulating immune responses against a specific disease causing micro-organisms (16).The cell wall of microbes acts as protective, structural shield and also contributes to interactive contacts with the human host during the initiation of infection and development (17). The types of immunity stimulated for protection are both innate and humoral responses.

The significant increase of mean percentage granulocytes in the

immunized groups in response to the challenge shows the roles of white blood cells in the fight against *Candida albicans* infections. This is because granulocytes, macrophages and monocytes are the first line defense mechanism recruited in the first few days of infection to engulf, digest and present pathogens for specific immune response (18). This increase in granulocytes for immunized albino rats is in line with the findings of Gow *et al.*, (19) that reported significant production of cytokines and chemokines which have phagocyte stimulatory properties.

However, the observable decrease of granulocytes in the infected group (positive control) is an indication of immunosuppression. This could be as a result of the ability of *C. albicans* to undergo switching or morphogenesis. These properties allow *C. albicans* to escape phagocytosis by piercing and subsequent killing of phagocytic cells leading to a decrease in circulating granulocytes (20). This finding is not in conformity with a study conducted by Jamalzadeh *et al.,* (21) which reported an increase in total white blood cells (neutrophils, basophils and eosinophils) in fungal infection of Caspian salmon.

The mid-range cells comprised of monocytes, eosinophils, basophils and immature cells (Abacus 380 automated blood analyzer manual). Therefore, the increase in mean percentage of mid-range cells in positive control as compared to the immunized and negative control groups may be attributed to the presence of increased amount of *C. albicans* in the blood to meet up with the burden. Therefore, could be because there is a continuous proliferation of immature cells in circulation (left shift)

Moreover, the significant increase in the percentage of lymphocytes in the immunized groups when compared to both controls could be because after immunization, the rats became sensitized and this result to the stimulation of memory cells to produce a substantial amount of lymphocytes (22, 23). The increase in lymphocytes is in conformity with a study conducted by Jamalzadeh *et al*., (21) which showed a significant decrease in fungal infection.

Clinical observations reported by Mathews and Burnie (24) indicated that antibodies play an important role in host defense against disseminated candidiasis because individuals with defects in cell mediated immune response are particularly prone to superficial but not disseminated candidiasis. Similarly, the significant ($P<0.05$) increase in the level of antibodies of the immunized rats shows that inactivated *C. albicans* has the potential of stimulating humoral immunity as evident in this study. The heat group produced a mean antibody titers of 1228.8µg/ml. This could be as a result of recognition of the immunogenic proteins and glycoproteins on the cell surface and subsequent stimulation of memory cells to produce significant quantity of antibodies on a second encounter of similar antigens (22, 23). Thus, this study correlates with findings of Cardenas-freytag *et al.*, (25) which reported a significant production of 3200 µg/ml immunoglobulins that were immunoprotective against candida infections in mice immunized with heat killed *C. albicans* in combination with adjuvant. Similarly, Thomas *et al.*, (15) also indicated a significant production of antibodies that are immune-protective during infection. In another study by Evron (13), the circulating antibodies in immunized mice that were immunoprotective were greater than 256µg/ml. Furthermore, in two separate

studies conducted by Saville *et al.*, (26, 27), showed vaccination with live attenuated *C. albicans* in mice challenged with a lethal dose of 5.2×10^6 virulent cells has a protective effect on candida infections. It is also interesting to note that Heat inactivated *C. albicans* produced more antibodies, lymphocytes and granulocytes as compared to the positive control. This could not be unconnected with the fact that β-glucans of live cells are normally masked by mannan-mannoprotein layer thus precluding recognition. However, the β-glucans of heat inactivated *C. albicans* is well exposed to recognition (19). This finding is in agreement with Gow *et al.*, (19) who reported that heat inactivated *C. albicans* induced significantly greater level of cytokines and chemokines which are known activators of immune cells.

Delayed-Type Hypersensitivity (DTH) reaction is initiated when antigens are presented by antigen presenting cells such as langerhans cells to sensitized memory T cells. The antigen presentation and subsequent T cell activation elicit an influx of macrophages, monocytes and lymphocytes at the site of antigen exposure. At the onset of DTH reaction, verso-permeability is increased so that additional cellular components migrate into the local site of antigen presentation (28).The observable swelling in the footpads of immunized group after 24 hours and 48 hours could be as a result of the responses by phagocytes at the site of infection. However, there was lesser swelling of footpads of the control group which received sterile normal saline during the same periods. The increase in the footpad sizes of the immunized group after 48 hours could be as a result of continued phagocytic activities in the footpads (25). This is in line with the findings of Evron (13) who reported a significant swelling of footpads to a maximum between 24 to 48 hours of challenge.

There is a 100% survival of rats in Heat inactivated as compared to the un-immunized infected rats where 100% death was recorded before the end of the experiment. This agrees with the report of Thomas *et al.*, (15). The mortality was caused probably by the breakdown in osmotic balance when the tissues were destroyed by the penetration of the hyphae and the lethargy that resulted from excessive energy exerted to overcome infection stress. Therefore, this result is also in agreement with the research conducted by Shah (29).

CONCLUSION

The findings of this study clearly indicated an interesting immune-protection of the immunized rats against *C. albicans* infections. It was also shown that systemic candidiasis can cause immune-suppression leading to mortality.

RECOMMENDATIONS

Further study should be conducted on freshly isolated C. albicans from patients heated with different temperature to determine the amount of heat suitable for maximum expression of immunogienic molecules.

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CONFLICT OF INTEREST

There is no conflict of interest in the course of the research.

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