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Evaluation of *in vivo* pathogenicity of *Candida* species isolated from palm wine and sorghum beer in a murine model

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ABSTRACT

Since *Candida* species frequently isolated in hospitals have been found in food processing environments, their presence in palm wine and sorghum beer may be of some clinical significance. This study was carried out to assess the *in vivo* pathogenicity of *Candida* strains isolated from these traditional beverages. Thus, ten potentially pathogenic strains were used to inoculate immunocompromised rat models by gavage and observed up to 30 days post-inoculation. On contrary to the control rats, the body weights of the animals inoculated with all the studied strains decreased over time, passing from 105-111 g to 89-98 g. Furthermore, the amount of white blood cells and platelets significantly increased while the red blood cell counts decreased, except those inoculated with *Kluyveromyces marxianus* strains. The blood smears taken at day-30 showed yeast cells in 60% of the inoculated rats. All strains in the study, except *Candida tropicalis* S10 and S13, were detected in the kidney with loads ranging from 2.58 to 7.24 log (CFU/g). In the liver, *C. tropicalis* S17, *K. marxianus* S87 and *K. marxianus* S2 were not detected. Thus, palm wine and sorghum beer yeast strains, mainly *C. rugosa, C. inconspicua* and *C. tropicalis* strains are capable of inducing candidemia.

Keywords: Body weight, haematological parameters, pathogen, rat model, traditional beverage, yeast strains.

INTRODUCTION

Palm wine and sorghum beer are traditional alcoholic beverages well appreciated by the population in Côte d'Ivoire. Thus, their consumption is getting more widespread and they constitute an important source of economic return for the producers, mainly for women. Furthermore, they contain a large amount of insoluble materials and have a nutritional value that significantly contributes to improve the diet of consumers (Aka et al., 2008). The fermentation process is mainly conducted by yeast strains among with *Saccharomyces cerevisiae* strains are the dominant. The study of genetic diversity showed that the ecological niche had a significant influence on *S. cerevisiae* population's native of Côte d'Ivoire and demonstrated a low heterozygosity (Tra Bi et al., 2019). The functions of *S. cerevisiae* in

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these beverages are related to the formation of alcohols and aromatics, the stimulation of lactic acid bacteria, improving nutritional value, inhibition of undesirable microorganisms and production of degrading tissue enzymes (Jespersen, 2003; Atter et al., 2021). The others yeast species reported are *Candida tropicalis, C. inconspicua, C. rugosa, C. parapsilosis, Kluyveromyces marxianus, Hanseniaspora guilliermondii, Rhodotorula mucilaginosa, Yarrowia lipolytica, Kodamaea ohmeri* and *Trichosporon asahii* (Tra Bi et al., 2016; Attchelouwa et al., 2018; Egue et al., 2018a; Amoikon et al., 2019). Some of these non-*Saccharomyces* species are regarded as opportunistic pathogens, particularly species belonging to the genus *Candida*.

Candida species are ubiquitous organisms and most of them are commensals, or at least transient commensals in the oral cavity, gastrointestinal tract and vagina. However, under certain circumstances, these species can become opportunistic pathogens causing candidiasis. In recent years, infections by these yeasts have become a significant health problem associated with the spread of Acquired Immunodeficiency Syndrome (AIDS), the increased use of the immunosuppressive therapy and the increase of nosocomial fungal infections (Upton and Marr, 2006; Diesse et al., 2017). The number of clinical infections worldwide has risen considerably in recent years with about 9.5 million HIV patients suffering from oral candidiasis (Menkem et al., 2015). *Candida albicans* is the most common and clinically relevant pathogen of the genus. However, there has been a significant trend in the emergence of species other than *C. albicans*, among which *C. tropicalis, C. glabrata, C. krusei, C. guilliermondii, C. parapsilosis, C. kefyr*, *C. dubliniensis* and *C. pelliculosa* (Diesse et al., 2017). Mycoses caused by *Candida* species show a wide spectrum of clinical presentations and can be classified as superficial, as with cutaneous and mucosal infections (oropharyngeal, oesophageal, vulvovaginal candidiasis), to deep, widespread and of high severity, as is the case with invasive candidiasis of internal organs (Segal and Frenkel, 2018). The latter are associated with high mortality rates ranging from 15 to 75% dependent on different patient groups and *Candida* species (Kullberg et al., 2011; Brown et al., 2012). The major pathogenetic mechanisms of invasive candidiasis include (1) disruption of the normal gastrointestinal microbiota which allows overgrowth of *Candida* species in the gut; (2) damage to the intestinal mucosal barrier which allows direct invasion of *Candida* cells into the bloodstream and abdominal cavity; and (3) impairment of the host immune response which allows overgrowth of *Candida* cells and dissemination into the bloodstream and subsequent organs, leading to deep-seated infections in various organs (Pappas et al., 2018).

Candida can contaminate humans through food. Indeed, *Candida* species frequently isolated in hospitals have also been found in food processing environments and have been identified as contaminants in a large number of foods. These foods include fruits and fruit juices, soft drinks, alcoholic beverages, food products with a high sugar content, vegetables and cereals, salted and acid-preserved foods, dairy products, meats and meat products (Cascio et al., 2007; Uhitil et al., 2009; Hutzler et al., 2012). Thus, the presence of these yeast species in palm wine and sorghum beer may be of some clinical significance. In our previous study, we showed by the *in vitro* tests that production of phospholipase, esterase, haemolysin and biofilm are the major virulence factors associated with *Candida* species and other potential pathogenic yeasts associated with palm wine and sorghum beer (Egue et al., 2018b). The aim of this study was to assess the *in vivo* pathogenicity of *Candida* strains isolated from these traditional beverages in order to contribute to their health quality.

MATERIALS AND METHODS

Strains and culture conditions

A total of ten strains were used in this study (Table 1). Three strains (*C. tropicalis* S115; *C. tropicalis* S17; *C. parapsilosis* S116)

were from clinical origin and used as control strains. They were isolated from AIDS patients and provided by the Centre for Diagnosis and Research on AIDS (CEDRES). The other strains were isolated from palm wine (*C. tropicalis* S10; *C. inconspicua* S20; *C. rugosa* S122; *Kluyveromyces marxianus* S87) and sorghum beer (*C. tropicalis* S13; *Trichosporon asahii* S127; *K. marxianus* S2). They belonged to the culture collection of the Food Technology Department (Nangui Abrogoua University, Abidjan, Côte d'Ivoire).

All the strains were identified by biochemical method (colony colour on *Candida* Chromogenic agar) and confirmed by molecular techniques (PCR-RFLP and sequencing the ITS1-5.8S-ITS2 region and D1/D2 domain) (Egue et al., 2018a). The yeast strains were maintained routinely at −20°C in 20% of glycerol. For experiments, stock cultures were plated on 1% yeast extract 1% peptone-2% glucose (YPD) agar and then incubated at 37°C for 24 h.

Animals and ethics statement

A total of 55 locally bred, 7-week-old female rats *(Rattus norvegicus*, type Wistar) weighting 100-130 g was used for the study. For the experiment, the animals were randomly assigned into eleven groups of five rats each and each group was housed in a separate metal cage. The rats were maintained in a conventional animal facility with 12 h light-dark cycle and allowed tap water and commercial-pelleted feed ad libitum. Rats were maintained under specific pathogen-free conditions within the animal care facility at the Department of Life and Earth Sciences (SVT) of the "Ecole Normale Supérieure de Côte d'Ivoire" and were allowed a one-week acclimatization period before experiments were started. This study complied with the tenets of the Declaration of Helsinki, and the animal experiments were approved by the Institutional Animal Care and Use Committee of Nangui Abrogoua University (CUSA-00125/2019-UNA).

Infection of animals

To prepare inocula, yeasts were grown in YPD medium at 37°C for 18 h at 180 rpm. Then, the cultures were centrifuged at 3000 rpm for 5 min and the pelleted cells washed twice in sterile phosphate-buffered saline (PBS) pH 7.2 and resuspended in a sterile saline to a final concentration of 0.5 OD (equivalent to 3.10^8 CFU/mL). Prior to infection, the rats were immunosuppressed by injection of cyclophosphamide (Endoxan, Bayër, France) (de Llanos et al., 2011). The rats were treated with 2 intraperitoneal injections of equal doses of cyclophosphamide on days 1 and 4 before infection (0.5 mL/100g animal body weight at the concentration of 200 µg/g). Each group of four rats was infected with appropriate inoculum: Groups 1 to 3 were respectively infected orally by gavage with 1 mL of clinical strains *C. tropicalis* S115, *C. tropicalis* S17 and *C. parapsilosis* S116. Rats from groups 4, 5, 6, 7, 8, 9, 10 received respectively *C. tropicalis* S10, *C. inconspicua* S20, *C. rugosa* S122, *K. marxianus* S87, *C. tropicalis* S13, *T. asahii* S127 and *K. marxianus* S2 orally. Negative control (Group 11) was inoculated with PBS orally. The animals were observed daily for up to 30 days post-infection. These animal experiments were conducted on two separate occasions to ensure reproducibility.

Parameters of evaluation

The weight of rats, the haematological parameters and yeast loads in rat organs were used to evaluate establishment of infection. The rats were weighed just before the start of the experiment at d0 corresponding to the day of infection by the yeast strains, one week after the infection (d7), two weeks (d14), three weeks (d21) and at the end of the study (d30).

Peripheral blood was collected from the retro-orbital plexus of each rat on d0, d7 and d30 after injection. Before blood collection, the animals were left to fast for 18 h. Approximately 0.5 mL of blood was placed in a sterile EDTA-anticoagulated tube and then taken to the laboratory for the hemogram using the MINDRAY BC 30S automatic haematology system (Shenzhen, China). In

addition, from the blood samples taken at d30, blood smears were taken and stained with Methylene Blue for yeast detection.

At the end of the experiment (d30), rats were humanely euthanized by cervical dislocation and kidney, liver and spleen were aseptically removed for yeast enumeration. The organs were weighed and homogenized in sterile PBS. Aliquots of 100 µL from serial 10-fold dilutions of organ homogenates were added to Sabouraud-Chloramphenicol agar, in duplicate. Plates were incubated at 37°C, and colonies were counted after 48 h. Results were expressed as numbers of colony-forming units (CFU) ± standard error per gram of tissue.

Statistical analysis

All statistical analyses were carried out using Prism V5.01 software (GraphPad Software, Inc., La Jolla, CA, USA) and the data were expressed as the means ± standard deviations (SD). The data were analysed with ANOVA One-Way. Dunnett's non-parametric t-test was used to compare the variance of the controls with that of the other groups. A P value < 0.05 was considered to be statistically significant.

Table 1: Yeast strains used in this study.

Strain	Origin	Pz index*				Biofilm*
		Aspartyl proteinase	Phospholipase	Esterase	Hemolysin	
Candida tropicalis S115	Clinical	0.91	0.48	0.44	0.45	18.50
Candida tropicalis S17	Clinical	0.91	0.48	0.50	0.21	23.90
Candida parapsilosis S116	Clinical	0.92	0.31	0.38	1.00	06.71
Candida tropicalis S10	Palm wine	0.90	0.32	0.36	0.40	22.70
Candida inconspicua S20	Palm wine	0.91	1.00	0.48	0.42	19.80
Candida rugosa S122	Palm wine	0.90	1.00	1.00	0.47	21.73
Kluyveromyces marxianus S87	Palm wine	0.92	0.49	0.47	0.46	13.91
Candida tropicalis S13	Sorghum beer	1.00	0.25	0.37	0.32	26.12
Trichosporon asahii S127	Sorghum beer	1.00	0.43	0.54	1.00	30.51
Kluyveromyces marxianus S2	Sorghum beer	1.00	1.00	1.00	0.41	06.70

* *In vitro* virulence factors were recorded based on the study of Egue et al. (2018b).

RESULTS

Yeast infection on rat body weights

Figure 1 shows the evolution of the weight of rats inoculated with the different yeast strains during the 30 days of experimentation. The average weight of the control rats increased during the experiment, from 103 to 122 g. On the contrary, the average weight of the inoculated rats decreased over the same period. Thus, the average weight of the rats inoculated with the clinical strains decreased from 107-111 g to 88.3-99.3 g. The highest decrease was observed in rats inoculated with *C. tropicalis* S17 (reduction of 18.7 g). When rats were inoculated with strains isolated from palm wine, the mean weights were between 89.3 and 98.3 g after 30 days of experimentation, i.e. a weight loss of 3.7 g, 12.7 g, 15.7 g and 17.5 g for rats inoculated with *C. rugosa* S122, *C. tropicalis* S10, *C. inconspicua* S20 and *K. marxianus* S87, respectively. With strains isolated from sorghum beer, the rats showed a similar mean weight of 93.3 g after 30 days of experimentation (reduction of 11.7-13.7 g).

Yeast infection on the rat haematological parameters

The amount of white blood cells in the blood of non-inoculated rats (control rats) increased very slightly during the experiment. The values increased from $5.1x10^3$ cells/µL to $6.4x10^3$ cells/µL (Table 2). On the other hand, in rats inoculated with strains isolated from palm wine and sorghum beer, the amount of white blood cells increased significantly. Thus, in rats inoculated with *T. asahii* S127 (Group 9), the values increased from $5.2x10^3$ cells/µL to $16.5x10^3$ cells/µL. In rats inoculated with the clinical strains, the count of white blood cells increased and then decreased. The values thus went from $5.4x10^3$ cells/µL (d0) to $17.1x10^3$ cells/µL (d7) and then to $13.8x10^3$ cells/µL (d30) in rats inoculated with *C. parapsilosis* S116.

The number of red blood cells decreased significantly in the rats inoculated with the strains isolated from palm wine and sorghum beer, except in the rats inoculated with *K. marxianus* (Groups 7 and 10) where it remained statistically identical and those inoculated with *C. rugosa* S122 (Group 6) where it increased at the end of the experiment (Table 2). In the rats inoculated with the clinical strains, the variations were different between the strains. Thus, the number of red blood cells increased in rats inoculated with *C. tropicalis* S17 ($4.7x10^6$ cells/µL to $6.7x10^6$ cells/µL) whereas it decreased and then increased in rats inoculated with *C. parapsilosis* S116.

As shown in Table 2, the amount of blood platelets increased in all rats included in the study. However, this increase was significant only in rats inoculated with *T. asahii* S127 (Group 9), *C. tropicalis* S10 (Group 4) and *C. tropicalis* S115 (Group 1).

Fungal burden of rat organs

Microscopic observation of the blood smears taken after 30 days of experimentation revealed the absence of yeast in the blood of the control rats and the presence of yeast cells in 60% of the inoculated rats (Figure 2). These rats were those inoculated with the strains *C. tropicalis* S115, *C. tropicalis* S13, *C. parapsilosis* S116, *C. inconspicua* S20, *C. rugosa* S122 and *K. marxianus* S2.

Enumeration showed any yeast cell in the different organs of the control rats (Table 3). However, all inoculated rats had yeast loads in at least one of the organs tested (liver, kidney, spleen). All strains in the study, except *C. tropicalis* S10 and S13, were detected in the kidney with loads ranging from 2.58 to 7.24 log (CFU/g). In the liver, strains of *C. tropicalis* S17, *K. marxianus* S87 and *K. marxianus* S2 were not detected. The strains detected showed loads between 2.16 and 7.34 log (CFU/g). In the spleen, only *C. tropicalis* S115 and *C. rugosa* S122 were detected. *Candida rugosa* had the highest loads [7.23-7.34 log (CFU/g)] and was found in all organs of the study. The clinical strain *C. tropicalis* S115 was also found in all organs.

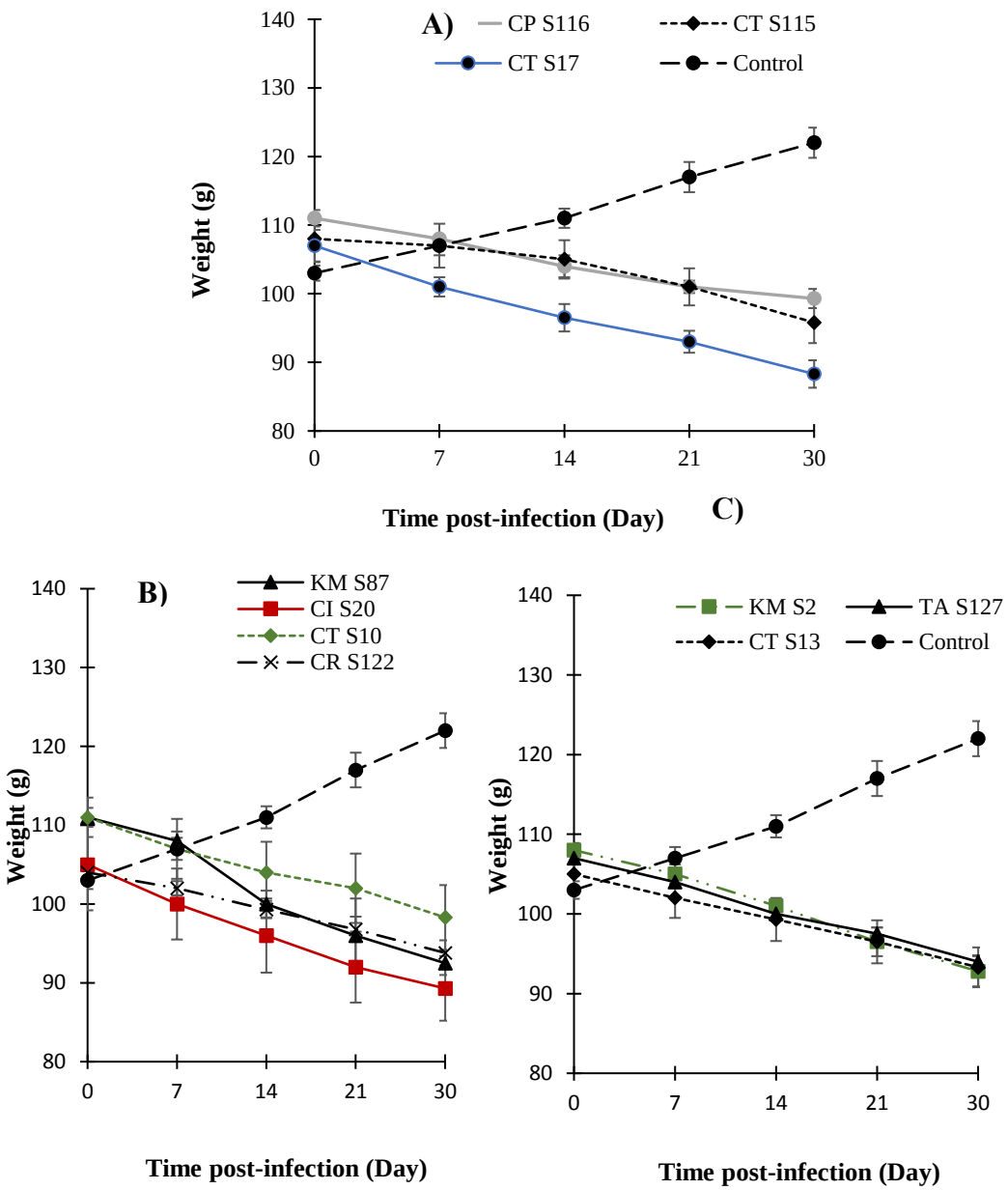

Figure 1: Weight of immunosuppressed rats after infection with different yeast strains.

The rats were treated with 2 intraperitoneal injections of equal doses of cyclophosphamide on days 1 and 4 before infection (0.5 mL/100g animal body weight at the concentration of 200 µg/g) and inoculated orally with 3.0 x 10^8 cells of each strains. The results are shown as the mean and the data are representative of two independent experiments (n = 5 rats). (A) = clinical strains; (B) = palm wine strains ; (C) = sorghum beer strains. CT = *Candida tropicalis*; CP = *C. parapsilosis*; CI = *C. inconspicua*; CR = *C. rugosa* ; TA = *Trichosporon asahii* ; KM = *Kluyveromyces marxianus.*

Table 2: Evolution of hematological parameters of immunosuppressed rats infected by yeast strains isolated from palm wine and sorghum beer in comparison to clinical strains.

Group of rats (inoculated strain)	Strain origin	White blood cells (10^3/µL)			Red blood cells (10^6/µL)			Blood platelets (10^3/µL)		
		d0	d7	d30	d0	d7	d30	d0	d7	d30
Group 1 (*C. tropicalis* S115)	Clinical	5.5±0.8^a	11.7±1.4^b	11.7±3.6^b	6.1±0.3^a	6.3±0.4^a	6.7±0.2^a	439±63.9^a	751±11.1^b	899±11.7^c
Group 2 (*C. tropicalis* S17)		4.0±0.5^a	13.5±1.2^b	9.5±1.4^b	4.7±0.3^a	5.4±0.6^{ab}	6.7±0.1^b	601±14.6^a	692±32.2^a	668±12.1^a
Group 3 (*C. parapsilosis* S116)		5.4±0.7^a	17.1±1.3^b	13.8±2.0^c	6.3±0.5^a	4.2±0.4^b	6.6±0.2^a	371±56.5^a	754±12.1^b	780±74.7^b
Group 4 (*C. tropicalis* S10)	Palm wine	4.6±0.6^a	7.0±0.7^a	8.8±0.5^a	5.3±0.2^a	3.3±0.1^b	4.3±0.1^b	551±94.7^a	751±11.4^b	823±13.1^c
Group 5 (*C. inconspicua* S20)		4.9±0.2^a	7.0±0.5^a	11.8±1.5^b	5.9±0.3^a	3.6±0.2^b	4.5±0.0^b	409±56.7^a	529±44.9^a	680±75.0^b
Group 6 (*C. rugosa* S122)		4.5±0.8^a	7.2±0.9^a	10.6±1.7^b	5.8±0.6^a	3.4±0.1^b	6.4±0.2^a	304±47.8^a	473±61.9^a	679±32.1^b
Group 7 (*K. marxianus* S87)		5.1±0.3^a	9.6±1.4^b	12.0±1.9^b	5.0±0.9^a	5.4±0.5^a	6.5±0.2^a	260±34.6^a	385±69.7^a	564±98.5^b
Group 8 (*C. tropicalis* S13)	Sorghum beer	4.5±1.1^a	9.1±1.9^a	13.0±1.5^b	6.3±0.5^a	4.0±0.2^b	4.8±0.2^b	583±50.1^a	659±12.2^a	823±94.2^b
Group 9 (*T. asahii* S127)		5.2±0.4^a	13.3±0.8^b	16.5±1.1^c	6.4±0.3^a	3.8±0.4^b	3.8±0.2^b	392±70.1^a	671±12.9^b	797±10.6^b
Group 10 (*K. marxianus* S2)		5.4±1.2^a	10.6±0.7^b	12.4±0.9^b	4.3±0.6^a	5.1±0.5^a	6.0±0.2^a	399±47.9^a	574±15.0^b	569±16.9^b
Group 11 (negative control)		5.1±0.3^a	5.3±0.2^a	6.4±0.2^a	5.6±0.1^a	5.9±0.4^a	6.4±0.2^a	412±76.1^a	627±10.2^b	718±39.2^c

The rats were treated with 2 intraperitoneal injections of equal doses of cyclophosphamide on days 1 and 4 before infection (0.5 ml/100g animal body weight at the concentration of 200 µg/g). The rats were inoculated orally with 3.0 x 10^8 cells of each strains and data were recorded on day d0 (corresponding to the day of infection), one week after the infection (d7) and at the end of the study (d30). The results are shown as the mean and the data are representative of two independent experiments (n = 5 rats). Different letters in the same column indicate significant differences ($p<0.05$).

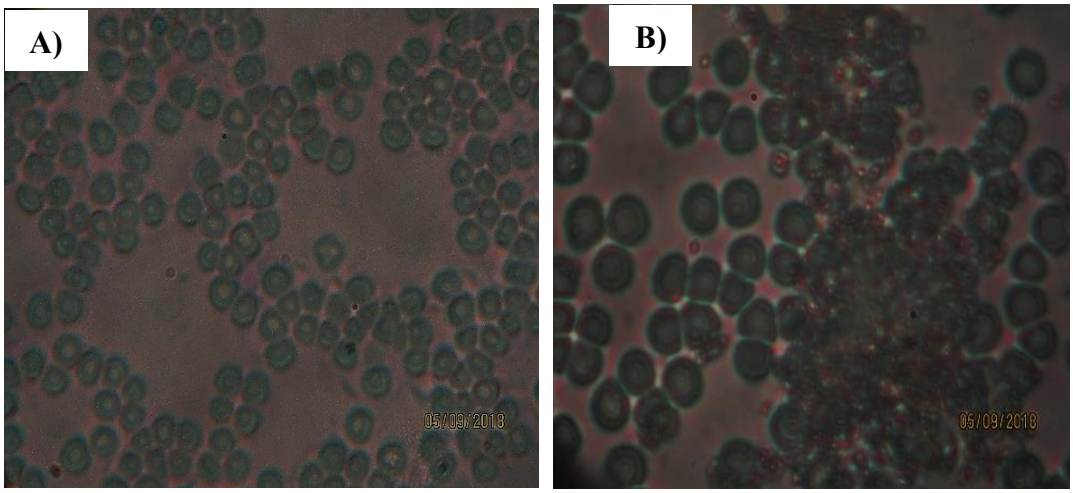

Figure 2: Blood smear showing the absence and the presence of yeast cells in the blood of control (A) and infected rats (B).

The rats were treated with 2 intraperitoneal injections of equal doses of cyclophosphamide on days 1 and 4 before infection (0.5 mL/100g animal body weight at the concentration of 200 µg/g). Blood smears were taken from blood sampled 30 days after inoculation and were stained with Methylene Blue.

Table 3: Yeast load in immunosuppressed rat organs after 30 days of inoculation (log CFU/g).

Group of rats (inoculated strains)	Kidney	Liver	Spleen
Group 1 (*C. tropicalis* S115)	4.88±0.05	3.47±0.79	3.30±0.65
Group 2 (*C. tropicalis* S17)	5.81±0.17	≤1	≤1
Group 3 (*C. parapsilosis* S116)	2.58±0.03	2.16±0.01	≤1
Group 4 (*C. tropicalis* S10)	≤1	4.19±0.06	≤1
Group 5 (*C. inconspicua* S20)	5.93±0.14	4.89±0.06	≤1
Group 6 (*C. rugosa* S122)	7.24±0.09	7.34±0.12	7.23±0.13
Group 7 (*K. marxianus* S87)	5.35±0.39	≤1	≤1
Group 8 (*C. tropicalis* S13)	≤1	4.44±0.86	≤1
Group 9 (*T. asahii* S127)	3.36±0.09	3.80±0.47	≤1
Group 10 (*K. marxianus* S2)	4.23±0.61	≤1	≤1
Group 11 (negative control)	≤1	≤1	≤1

The rats were treated with 2 intraperitoneal injections of equal doses of cyclophosphamide on days 1 and 4 before infection (0.5 mL/100g animal body weight at the concentration of 200 µg/g). The rats were inoculated orally with 3.0 x 10^8 cells of each strains and data were recorded 30 days after inoculation. The results are shown as the mean and the data are representative of two independent experiments (n = 5 rats).

DISCUSSION

The study of human infectious diseases requires the investigation of microorganisms in model systems. Here, immunocompromised rat models were used to study the pathogenic effect of yeast strains isolated from palm wine and sorghum beer. As key risk factors for developing candidemia in humans include neutropenia, mucositis, and the use of broad-spectrum antibiotics (Hirayama et al., 2020), cyclophosphamide was used to induce neutropenia. Furthermore, the animals were inoculated by gavage (intragastrically) and the pathogenic effect was highlighted by the yeast strains influence on the body weight, white blood cell, red blood cell, blood platelet counts as well as yeast load in different organs of these animals. On contrary to direct injection of *Candida* cells via the tail-vein which is the most route used in murine model, intragastrically infection mimicked step of translocation from the gut (Hirayama et al., 2020).

The weights of the animals inoculated with all yeast strains decreased over time compared to the weights of the control rats. The change may imply that food nutrient absorption and utilization were affected (Adigwe et al., 2021). This decrease could be due to the development of an infection following the multiplication of the inoculated strains. Such an infection would cause a dysfunction of the vital organs of these rats.

Haematological assessment in animals is of capital interest in defining the diagnosis of many diseases. Alteration of these haematological parameters (white blood cells, red blood cells and platelets) is an indicator of general system dysfunction in animals (Vinodini et al., 2015). The results showed a significant decrease in red blood cells in rats inoculated with strains isolated from palm wine and sorghum beer, except *K. marxianus* strains (S2 and S87). These results demonstrated a disturbance of haematological parameters from the day 7 post-inoculation, which proves that the yeast strains of the study have the capacity to alter cellular functions and induce a pro-inflammatory response in the rats. Moreover, this decrease in red blood cells is in accordance with previous study which demonstrated the haemolytic activity of yeast strains isolated from these traditional beverages, except *T. asahii* strains (Egue et al., 2018b). In fact, the strains secreted haemolysins which are pore-forming toxins capable of lysing red blood cells, releasing iron needed as a growth factor by several fungi. According to Tsang et al. (2007), haemolysin secretion followed by iron acquisition facilitates deeper tissue invasion by *Candida* species. The presence of yeast strains such as *C. inconspicua* S20, *C. tropicalis* S10, *T. asahii* S127 and *C. tropicalis* S13 on palm wine and sorghum beer consumers would therefore constitute a health risk, which is manifested by a decrease in the number of red blood cells, and consequently anaemia.

In terms of the leucocyte formula, the results also showed a significant increase in the amount of white blood cells compared to the control rats. The increase in these blood cells is indicative of a general inflammatory state (Fahim et al., 2012) which would be due to the damage caused by the presence of yeast strains. This could be explained by the stimulation of the immune system leading to these increases (Enuneku and Ezemonye, 2013). According to Teeter and Franciscus (2004), high white blood cell counts in humans indicate that the body is fighting an infection. The results of this study corroborate those of Demenesku et al. (2014) who found a significant increase in blood leukocytes compared to the control in rats treated by intra-peritoneal injection at 1 mg/kg for 48 hours with Cadmium.

An increase in blood platelets was also observed in rats inoculated during the study period. This increase was observed in rats inoculated with the clinical strains and also in those inoculated with the strains from the analysed beverages especially in rats inoculated with *T. asahii* S127, *C. tropicalis* S10, *C. tropicalis* S13 and *C. inconspicua* S20. Blood platelets play a role in the blood clotting process when tissue is damaged. An increase in platelets may thicken the blood and thus change the viscosity of the plasma (Pekkanen et al., 2000; Jarup, 2003).

Although the blood smears revealed the presence of yeast cells in 60% of rat groups

under study, all inoculated rats had yeast loads in at least one of the organs tested (liver, kidney, spleen). This may indicate the differences in pathogenicity among *Candida* strains of the study. According to the yeast cell counts in organs of infected rats, *C. rugosa* S122 and *C. inconspicua* S20 were the most pathogenic strains. The different cell count found here imply clearly that the colonisation potential is an intrinsic ability of each *Candida* spp. and may vary between isolates within a given species. Furthermore, most of animals had kidney infection. This result confirms the affirmation that kidney is usually the principal target organ for disseminated candidiasis. The presence of yeast in rat organs implies that the yeasts would have overcome the natural defence barriers of the rats to settle and reproduce in the organs studied. This could lead to tissue damage to these organs. In fact, the microorganisms, by accumulating in the kidneys, inhibit erythropoietic activity by damaging the synthesis of erythropoietin, a hormone secreted by the kidneys whose role is to stimulate red blood cells (Diaby et al., 2016).

Conclusion

According to the results of the present study, it could be concluded that palm wine and sorghum beer yeast strains, mainly *C. rugosa, C. inconspicua* and *C. tropicalis* strains are capable of inducing candidemia. The infection may lead to invasive candidemia with the kidney and liver as the principal target organs. People who may have an increased risk include consumers who have a weakened immune system (for example, from being extremely ill or receiving chemotherapy), who have taken broad-spectrum antibiotics, diabetics or with very low neutrophil count. Since candidemia diagnosis can be difficult, there is a great need to apply more hygienic conditions during handling, storage and/or selling these beverages.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

LANE contributed to the data curation, formal analysis & validation. MG contributed to the methodology, supervision, validation, writing-review & editing. FKN worked on the conceptualization, the data curation, the formal analysis, methodology, writing-original draft, writing-review & editing. MKC contributed to the conceptualization, methodology & provided supervision.

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