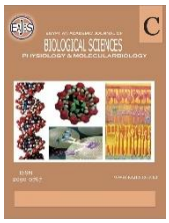
.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 16(2) pp89-94 (2024)*
DOI: 10.21608/EAJBSC.2024.372334

Egypt. Acad. J. Biolog. Sci., 16(2):89-94 (2024)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Assessed the Liver Injury Biomarker Following Candida Infection in Female Diabetic Rats

Marwa M. Mohammed[1]; Mohamed Abd Al-Razek[2]; Zohour I. Nabil[1] and Heba N. Gad El-Hak[1*]

[1]Zoology Department, Faculty of Science, Suez Canal University.
[2]Botany Department, Faculty of Science, Suez Canal University.
***E-mail:** Heba_nageh@hotmail.com

ARTICLE INFO
Article History
Received:3/7/2024
Accepted:7/8/2024
Available:11/8/2024

Keywords:
Vaginal Candida; infection; liver; biochemical; AST.

ABSTRACT

Candidasis, an important cause of morbidity and death, has been on the rise globally in the last several decades, particularly among very sick patients. Because of its immunosuppressive effects, the metabolic disease diabetes mellitus (DM) makes patients more likely to contract fungal infections, such as those caused by Candida sp. Only a small number of studies have established a causal relationship between vaginal Candida infection and liver injury. Researchers in this study looked at how a vaginal Candida infection affected a biomarker for liver damage in Wistar rats. The researchers formed two groups of ten female Wistar albino rats each, out of a total of twenty. We used rats that did not undergo any treatment as controls. The female rats in the infected group will receive an intraperitoneal injection of 120 mg/kg of alloxan to suppress their immune system. The next step is to confirm that the glucose levels are high. Next, the vaginal opening is inoculated with the isolated yeast *C. albicans* at a concentration of approximately 5 x 10^8 and allowed to grow for 5 weeks. They had their blood tested for total bilirubin, total protein, alanine aminotransferase, and aspartate aminotransferase. Disturbed and distinct from the control group were the liver injury biomarkers. Finally, vaginal Candida infections damage the livers of Wistar rats.

INTRODUCTION

Hyperglycemia characterizes diabetes mellitus (DM), a metabolic and degenerative disease that accelerates macro- and micro-vascular changes and causes long-term complications such as retinopathy, neuropathy, and nephropathy (Uma Devi, 2007). It is quickly becoming the most significant new danger to public health in the modern era (Tulchinsky and Varavikova, 2010). Changes in polymorphonuclear cells, monocytes, and lymphocytes, as well as a general weakening of cellular immunity, are among the system abnormalities associated with diabetes (Daryabor *et al.*, 2020).

Among elderly patients with candidemia, diabetes mellitus (DM), heart disease (HD), and lung disease (LPD) are more common (Extremera *et al.*, 2015). Numerous studies have examined the link between diabetes and candidiasis (Lamster *et al.*, 2008). This is mainly because people with diabetes are more likely to get fungal infections than those without the disease (Casqueiro *et al.*, 2012).

Depending on whether the infection is localized, or systemic, various mechanisms contribute to a higher *Candida sp.* predisposition in diabetes patients (Pozzilli and Leslie, 1994). Certain host conditions can facilitate candidal colonization and infection. These include yeast adhesion to epithelial cell surfaces, increased glucose levels in saliva, decreased salivary flow, microvascular degeneration, and impaired neutrophil candidacidal activity (Mohammed *et al.*, 2021). When combined with glucose, the release of multiple degradative enzymes, or a patient's overall immunosuppression state, these conditions take on an even more alarming tone. All these things tip the scales in favor of Candida sp., making it easier for them to go from commensal to pathogen and infect hosts (Miramón and Lorenz, 2017). Diabetic patients have a higher prevalence of intestinal Candida albicans colonization, according to a recent study by Gürsoy *et al.* (2018). The previous work on infected rats subjected to stress for 10 days revealed significant changes in their immune response against Candida albicans (Rodriguez-Galán *et al.*, 2003). Increased fungal colonization and lesions associated with acute steatosis, consisting of lipid accumulation in the cytoplasm of hepatocytes, were observed in the livers of stressed and infected rats after 3 days of treatment (Schaarschmidt *et al.*, 2018). This study investigated the impact of a vaginal Candida infection on a biomarker associated with liver damage in female Wistar rats.

MATERIALS AND METHODS

Animals:

Seven days before the start of the experiments, the experimental room was filled with outbred female Wistar rats weighing 100-150 g. The female rats were kept at 22^0C and subjected to a 12-hour light-dark cycle with constant access to water and food. All procedures were greenlit by the Scientific Research Ethics Committee of the Faculty of Science at Suez Canal University in Ismailia, Egypt.

Experimental Design:

Rats were assigned to two groups: Normal uninfected and C. albicans-infected (Ca) groups. Rats were infected after induction of diabetes by alloxan, Then, isolated yeast *C. albicans* will be inoculated in concentrations of about 5 $x10^8$ in the vaginal opening and left to grow for 5 weeks according to Carrara *et al.* (2009). Blood was obtained for biochemical assays.

Biochemical Analysis:

The blood samples were centrifuged, serum aspirated and analyzed for Bilirubin concentrations, blood Alanine aminotransferase (ALT), blood Aspartate aminotransferase (AST), total Protein concentration, albumin and globulin concentration using commercial kits.

Serum bilirubin was determined using the commercial kit (Diamond, Diagnostics company, Egypt, recommended by REF: 265 ml) according to the quantitative determination of bilirubin method, (Šuk *et al.*, 2019).

In accordance with Fernández-Real *et al.* (2010) a commercial kit (SPINREACT company, Spain, REF: SP41274) was used to determine serum ALT. Alanine was changed into glutamate and pyruvate by adding an amino group to α-ketoglutarate in a way that can be undone. ALT helped make this happen. The created pyruvate is then converted into NADH and lactate-by-lactate dehydrogenase (LDH). A sample's catalytic concentration of ALT determines the rate of reduction in NADH concentration as measured photometrically.

Following the protocol laid out by Stavreva Veselinovska (2016), AST in serum was measured using a commercial kit from SPINREACT in Spain (Ref: MD41264). AST helped move an amino group from aspartate to alpha-ketoglutarate in a way that could be undone to make glutamate and oxalacetate. Reducing the oxalacetate to malate-by-malate Dehydrogenase (MDH) and NADH is the process that is carried out. The photometric rate of decrease in NADH concentration is directly proportional to the catalytic concentration of AST in the sample.

According to Guobing *et al.* (2001), a commercial kit (Vitroscient, Egypt, REF: 13501) was used to determine serum total protein using the Biuret colorimetric endpoint method. The biuret reaction is the basis of the total protein reagent. In this reaction, divalent copper forms the pink-to-purple biuret complex with the peptide bonds of protein under alkaline conditions. A specimen's color intensity is proportionate to its total protein concentration. It is calculated by tracking the rise in absorbance between 530 and 570 nm.

A commercial kit from Vitro Scient in Egypt (REF: 1011) was used to determine serum albumin using the colorimetric diazo method, as per the protocol outlined by (Yang *et al.,* 2018). Thanks to the development of dye binding techniques, albumin measurement has become much easier. By lowering the pH of the reaction, the bromocresol green (BCG) method is better at binding dyes because it is more specific, sensitive, and does not interfere with other colors. The anionic dye bromocresol green (BCG) can be bound to albumin at pH 4.2, making the resultant complex blue-green in color: Albumin + BCG →Albumin-BCG complex. The concentration of albumin in the specimen is directly proportional to the intensity of the blue-green color. It is calculated by tracking the rise in absorbance between 580 and 630 nm.

RESULTS

Total bilirubin, direct bilirubin, ALT, AST, total protein and albumin in the control and infected group with Candida sp. was demonstrated in Table 1. Data revealed that total bilirubin, direct bilirubin ALT, AST, total protein and albumin significantly $p \leq 0.05$ in the infected group in comparison to the control group.

Table 1: Effect of infection with *Candida albicans* on the liver injury biomarker of the female rats

Parameter	Groups	
	Control	Infection
	Mean ±S.E	Mean ±S. E
Total bilirubin (mg/dL)	0.93±0.07	1.80±0.15*
% of change		93.5%
Direct bilirubin (mg/dL)	0.37±0.04	0.98±0.04*
% of change		164.8%
ALT (U/L)	35.1±1.88	69.6±3.02*
% of change		98.2%
AST (U/L)	34.5±1.52	65.3±2.69*
% of change		89.2%
Total protein (g/dL)	5.68±0.10	6.82±0.24*
% of change		20%
Albumin (g/dL)	4.63±0.19	5.28±0.16
% of change		14%

Data were expressed as means ± SEM, n=10. Data were statically analyzed using T- test. (*) showed data which is statistically significant at $P \leq 0.05$.

DISCUSSION

The liver is susceptible to xenobiotic-induced damage due to its central role in xenobiotic metabolism and its position as a gateway in the circulatory system (Österreicher *et al.*, 2012). Damage to the liver may have occurred due to changes in liver function (Chao *et al.*, 2016). One reliable measure of liver function is the total protein, albumin,

globulin, direct and indirect bilirubin, ALT, and AST levels (Papadia e*t al.,* 2004). In this study, compared to the control group, participants with candida infections had significantly higher levels of direct bilirubin, indirect bilirubin, and total bilirubin. Bilirubin levels that are too high could be a sign of liver disease or rapid hemolysis (Hu *et al.,* 2013). According to Gowda *et al.* (2009), hepatic enzymes ALT and AST can be used as biochemical indicators of early acute hepatic damage. The transamination reactions and serum activities of the ALT and AST enzymes involved in amino acid metabolism will likely get stronger because of damage and leakage in the cell membranes. These enzymes are localized in the periportal hepatocytes (Adedara e*t al.,* 2010). In this study, Candida infection was found to cause a notable rise in the serum enzyme activities that indicate liver function. The higher enzyme activities seen in the groups treated with candida may be because the yeast worked with these signs of liver damage to make them stronger. Similarly, Pargaputri and Andriani (2021) reported that rats infected with candida displayed significantly higher elevations in ALT and AST levels. One possible explanation for the rise in hepatic enzymes is that the liver was damaged due to the toxic effects of a candida infection.

The infected group exhibited a statistically significant rise in total protein data ($P \leq 0.05$). The infected group's total protein level was elevated because stress leads to abnormal protein accumulation (Juan *et al.,* 2021). Medicated serum In the infected group, albumin and globulin levels were significantly higher ($p \leq 0.05$). according to O'Connell *et al.* (2005), infections and diseases can cause albumin and globulin levels to rise.

CONCLUSION

It is evident that vaginal infection with candida albicans induced liver injury. It is recommended to analyze liver function regularly during vaginal infection of candida and be in consideration during the fungal treatment**.**

Declarations:

Ethical Approval: Not applicable

Conflict of interests: The authors declare no conflict of interest.

Authors Contributions: All authors contributed equally, and have read and agreed to the published version of the manuscript.

Funding: No funding was received.

Availability of Data and Materials: The data underpinning the findings of this study are accessible upon request from the corresponding author.

Acknowledgements: Not applicable

REFERENCES

Adedara, I., Owumi, S., Uwaifo, A., and Farombi, E. (2010). Aflatoxin B1 and ethanol co-exposure induces hepatic oxidative damage in mice. *Toxicology and Industrial Health, 26*, 717-724.

Carrara, M.A., Bazotte, R.B., Donatti, L., Svidzinski, T.I., Consolaro, M.E., Patussi, E.V., and Batista, M.R. (2009). Effect of experimental diabetes on the development and maintenance of vulvovaginal candidiasis in female rats. *American Journal of Obstetrics and Gynecology, 200*, 659.e651-654.

Casqueiro, J., Casqueiro, J., Alves, C.J.I.j.o.e., and metabolism (2012). Infections in patients with diabetes mellitus: *A review of pathogenesis, 16*, S27-S36.

Chao, C.-Y., Battat, R., Al Khoury, A., Restellini, S., Sebastiani, G., and Bessissow, T. (2016). Co-existence of non-alcoholic fatty liver disease and inflammatory bowel disease: A review article. *World journal of gastroenterology, 22*, 7727.

Daryabor, G., Atashzar, M.R., Kabelitz, D., Meri, S., and Kalantar, K.J.F.i.i. (2020). The effects of type 2 diabetes mellitus on organ metabolism and the immune system. *11*, 546198.

Extremera, B.G., López, P.J., Ramírez, A.J.G., Peñalver, E.G., Martínez,

M.L.A., and Fernández, I.M. (2015). Clinical Trials on Diabetes Mellitus. In Treatment of Type 2 Diabetes (IntechOpen). 5, 119-143.

Fernández-Real, J., Ortega, F., Gómez-Ambrosi, J., Salvador, J., Frühbeck, G., and Ricart, W. (2010). Circulating osteocalcin concentrations are associated with parameters of liver fat infiltration and increase in parallel to decreased liver enzymes after weight loss. *Osteoporosis International*, *21*, 2101-2107.

Gowda, S., Desai, P.B., Hull, V.V., Math, A.A.K., Vernekar, S.N., and Kulkarni, S.S. (2009). A review on laboratory liver function tests. *The Pan african medical journal*, 3:17.

Guobing, X., Lili, J., Lihua, Z., and Tiean, X. (2001). Application of an improved biuret method to the determination of total protein in urine and cerebrospinal fluid without concentration step by use of Hitachi 7170 auto-analyzer. *Journal of clinical laboratory analysis*, *15*, 161-164.

Gürsoy, S., Koçkar, T., Atik, S.U., Önal, Z., Önal, H., and Adal, E.J.K.j.o.p. (2018). Autoimmunity and intestinal colonization by Candida albicans in patients with type 1 diabetes at the time of the diagnosis. Korean Journal of Pediatrics, *61*, 217.

Hu, Z., Sun, Y., Wang, Q., Han, Z., Huang, Y., Liu, X., Ding, C., Hu, C., Qin, Q., and Deng, A. (2013). Red blood cell distribution width is a potential prognostic index for liver disease. *Clinical Chemistry and Laboratory Medicine*, *51*, 1403-1408.

Juan, C.A., Pérez de la Lastra, J.M., Plou, F.J., and Pérez-Lebeña, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International Journal of Molecular Sciences*, *22*, 4642.

Lamster, I.B., Lalla, E., Borgnakke, W.S., and Taylor, G.W.J.T.J.o.t.A.D.A. (2008). The relationship between oral health and diabetes mellitus. The Journal of the American Dental Association, *139*, 19S-24S.

Miramón, P., and Lorenz, M.C.J.P.p. (2017). A feast for Candida: metabolic plasticity confers an edge for virulence. *PLOS Pathogens*, *13*, e1006144.

Mohammed, L., Jha, G., Malasevskaia, I., Goud, H.K., and Hassan, A.J.C. (2021). The interplay between sugar and yeast infections: do diabetics have a greater predisposition to develop oral and vulvovaginal candidiasis? *Cureus Journal of Medical Science*, *13*(2):e13407

O'Connell, T., Horita, T.J., and Kasravi, B. (2005). Understanding and interpreting the serum protein electrophoresis. American Family Physician, *71*, 105-112.

Österreicher, C.H., Trauner, M.J.E.o.o.d.m., and toxicology (2012). Xenobiotic-induced liver injury and fibrosis. Expert Opinion on Drug Metabolism and Toxicology, 8, 571-580.

Papadia, F.S., Marinari, G.M., Camerini, G., Murelli, F., Carlini, F., Stabilini, C., and Scopinaro, N. (2004). Liver damage in severely obese patients: a clinical-biochemical-morphologic study on 1,000 liver biopsies. *Obesity surgery*, *14*, 952-958.

Pargaputri, A.F., and Andriani, D. (2021). Hepatocellular Liver Function of Immunosuppressed Rats with Oral Candidiasis after Hyperbaric Oxygen Treatment: Alanine Transaminase and Aspartate Transaminase Levels. *Archives of Orofacial Science*, *16*.:5-9.

Pozzilli, P., and Leslie, R.J.D.M. (1994). Infections and diabetes: mechanisms and prospects for prevention. Diabetic Medicine, *11*, 935-941.

Rodriguez-Galán, M.C., Sotomayor, C., Costamagna, M.E., Cabanillas, A.M., Rentería, B.S., Masini-Repiso, A.M., and Correa, S.J.A.J.o.P.-C.P. (2003). Immunocompetence of macrophages in rats exposed to Candida albicans infection and stress. *American Journal of Physiology-Cell Physiology, 284*, C111-C118.

Schaarschmidt, B., Vlaic, S., Medyukhina, A., Neugebauer, S., Nietzsche, S., Gonnert, F.A., Rödel, J., Singer, M., Kiehntopf, M., and Figge, M.T.J.T. (2018). Molecular signatures of liver dysfunction are distinct in fungal and bacterial infections in mice. *Theranostics, 8*, 3766.

Stavreva Veselinovska, S. (2016). The activities of the aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase enzymes in the blood serum of rats in conditions of chronic lead poisoning. Paper presented at: Seminar Of Ecology With International Participation–2015, Section "Biology" (Union Of Scientist In Bulgaria).

Šuk, J., Jašprová, J., Biedermann, D., Petrásková, L., Valentová, K., Křen, V., Muchová, L., and Vítek, L. (2019). Isolated silymarin flavonoids increase systemic and hepatic bilirubin concentrations and lower lipoperoxidation in mice. *Oxidative medicine and cellular longevity, 6026902, 1-12*.

Tulchinsky, T.H., and Varavikova, E.A.J.P.h.r. (2010). What is the "new public health"? *Public Health Reviews, 32*, 25-53.

Uma Devi, T. (2007). Diabetes Mellitus Type 2: Evaluation of Microvascular and Macrovascular Complications (Stanley Medical College, Chennai). PhD. thesis.