International Journal of Educational Research and Development
www.journalofeducation.in
Online ISSN: 2664-7095; Print ISSN: 2664-7087
Received: 01-11-2019; Accepted: 02-12-2019; Published: 07-01-2020
Volume 2; Issue 1; 2020; Page No. 01-04

Effects of ethanolic extract of mustard seed on kidney tissues of albino rats infected with candida SP

Enas Abd-El Hay Taha Tolba
Faculty of Science; Jazan University, Biology Department, Saudi Arabia

Abstract
Mustard seeds (Brassica species) are widely used as medicinal crops and spices. A study designed to investigate the possible renal protective effect of Mustard seed (MS) extract was assessed against induced candida infection in albino rats. Animals were divided into 4 groups, each of 5 rats. Control group receive distilled water by oral gavage; the second group treated with ethanolic extract of Mustard seed (500 mg/kg body weight) orally every day for 14 days. The third represents candida infected animals. The forth group represented the infected animals treated with mustard seed extract. Elevated serum renal biomarkers such as urea and creatinine were observed due to candida infection. Combined administration of mustard seed extract with candida infection to rat partially normalized the altered biochemical markers. Moreover, fungal infection caused histological changes in kidney of rat including cloudy swelling of renal tubules as well as, atrophied glomerulus. However, co-administration of mustard seed extract with infection alleviated to some extent the changes and the kidney structure and function return normal state. The results of this study strongly indicate that Mustard seed has possible potent renal protective action candida infection against in rats.

Keywords: kidney injury, candida infection, mustard seed extract, rat

Introduction

Funguria is common in hospitalized patients due to invasive infection of the kidney. The vast majority of fungal infections of the kidney and bladder result from Candida albicans and other Candida species (Kullberg and Arendrup, 2015) [10]. Despite advances in antifungal therapy, the mortality associated with invasive candidiasis remains as high as 40% (Olivas-Escárcega *et al*., 2008) [14]. Current choices for treatment include fluconazole, caspofungin, voriconazole, and amphotericin B (Pappas *et al*., 2004) [15]. As recorded by Myoken *et al*. (2004) [13] retrospective analysis of fungemia in patients with hematologic malignancies revealed that four patients, who received fluconazole and itraconazole during neutropenia, developed breakthrough candidemia due to azole-resistant *Candida tropicalis* isolates. Which indicated that there were risk for drug resistance for this reasons there must be a serious effort for discovering new drugs for treating candidiasis.

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine (Chitme *et al*., 2004) [5]. About 80% of the people in developing countries use traditional medicines for their health care (Kim, 2005) [8]. Mustard seed is a well-known medicinal plant in traditional medicinal system and new scientific studies have highlighted the possible use of mustard seed in modern medicine (Amarowicz *et al*., 1996) [1]. The aim of the present study was to evaluate the renal protective activity of mustard seed extract against pathogenic fungus Candida spp. The plants were selected on the basis of their reported ethnobotanical uses.

Materials and Methods

Experimental animals

Rats were obtained from the Animal House of Zoology Department, Faculty of Science, Suez Canal University. They were housed in plastic cages (5 rats in each cage) with bedding under natural laboratory conditions and on light and dark cycle (LD). They were allowed free access to food, water (ad libitum) and were maintained for one week in the experimental room habituation.

Preparation of seed extracts

The seeds were grounded to a fine powder using a clean mortar and pestle, their powder was stored in a clean bottle at room temperature in dark place. The powdered seed samples (100g) were weighed by using a sensitive balance and extracted with 300 ml of 70% ethanol. Then shacked for about 3 days. The extracts were filtered through Whatman No. 1 filter paper followed by evaporation of alcohol. The extract were transferred to glass vials which stored at 4º C until using (Amarowicz *et al*., 1996) [1]

Experimental design

Twenty male albino rats, aged three months and weighing 100 - 140g were divided into four groups of five rats. Group I: The rats were recieve orally distilled water once daily for 14 days. Group II: The rats were injected orally with Mustard seed extract in a dose of 500 mg/kg bw once daily for 10 consecutive days**.** Group III: infected group; intraperitoneally challenged with 1000 µL of the *Candida sp* suspension (2×10^6 cell/mL). Group (IV): infected animals and treated with mustard seed Extract (500mg/kg; orally). Animals were treated 72 hours post- candida infection.

Blood sampling

At the end of the experiment, rats were anaesthetized with light diethyl-ether. The blood sample were obtained directly from the retro orbital vein. The collected blood, were allowed to stand in

slanting position for about 45 minutes at 4°C.The serum was separated by centrifugation at 4000 rpm for 15 min. The obtained serum was used to estimate the intended biochemical parameters.

Determination of serum total Protein

Protein molecules contain a large number of peptide bonds. When treated with copper ions (Cu^{+2}) in alkaline solution, a colored complex is formed between the copper and the carbonyl and the amine groups of theses peptides. As a similar reaction occurs with biuret (the simplest of such compounds formed by heating urea), the term "biuret reaction" was adopted.

The method described is based on the reports of Weischselbaum (1946). The violet color developed is proportional to the number of peptide bonds in the protein and is nearly independent of the relative concentration of albumin and globulin.

Determination of serum albumin

Serum albumin was measured using commercially available kit provided by Stanbio, San Antonio, TX, USA. According to the method presented by Doumas *et al*. (1971) [6] was modified by using citrate instead of succinate buffer, a lower buffer concentration and reading of the final color at 550 instead of 630 nm.

Determination of serum urea level

Serum urea level was determined using commercial kit (Biomed diagonestic, Hannover, Germany) according to manufacturer's instruction. Based on the method of Vassault *et al.* (1986) [1], urea is hydrolyzed by ureases forming ammonia and carbamic acid. Carbamic acid spontaneously decomposes into ammonia and carbon dioxide. Then, in the presence of salicylate and nitroferricyanide, ammonia reacts in alkaline solution of sodium hypochlorite, to form a green dye compound. The intensity of the green dye was measured by UV- spectrophotometer at wave length 578nm at room temperature which is directly proportional to the amount of urea concentration. Serum urea level was expressed as mg/dl. Rats normal range (15-20 mg/dl).

Urea= ((A) sample / (A) stander) × 50 (A): Absorbance

Determination of serum creatinine level

Serum creatinine level was determined using commercial kit (Bio-diagenostic, Egypt) according to the colometric methods of Bartels *et al.* (1972). Creatinine reacts with picric acid in alkaline solution (sodium hydroxide) to form a colored complex. The intensity the complex formed directly proportional to creatinine concentration and measured by UV-spectrophotometer at wave length 495nm at room temperature. Serum creatinine level was expressed as mg/dl. Rats' normal range (1.09-1.7 mg/dl).

Creatinine= ((A) sample / (A) stander) × 6 (A): Absorbance

Sampling for Histopathology Preparations

After dissection of animal's kidney samples were immediately fixed into 10% formalin at ambient temperature up to 24 hours. Then the tissue was treated for blocks. Tissue samples were sectioned at 5 µm and stained with hematoxylin and eosin (HE) or Masson's Trichome stain for histological examination using optical microscope according to the method described by Langenberg *et al*. (2008) [11].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) computer software version 20 was used for data analysis. The results of the tests were analyzed using One Way Analysis of Variance at 95% confidence interval followed by Duncan multible range test; with $p<0.05$ being considered as significant.

Results

Effect of Mustard seed extract on serum albumin (ALB)

The levels of serum albumin in infected and treated groups is graphically demonstrated in Figure (1). One-way analysis of variance was conducted to determine whether samples means are significantly different from each other. The analysis indicated that there was significant (P <0.05) decrease found in serum of candida infected group when being compared to the control group. After 2 weeks of mustard extract treatment, the level of serum albumin was significantly increased as compared with infected group.

Effect of Mustard seed extract on serum Total protein.

Figure (2) represents the level of total protein after treatment with tested extract in control and infected animals. The current results indicated that candidia infection results in significant decrease in total protein as compared with control group. However, treating infected animals with mustard seed extract resulted in significant elevation in total protein as compared with infected groups.

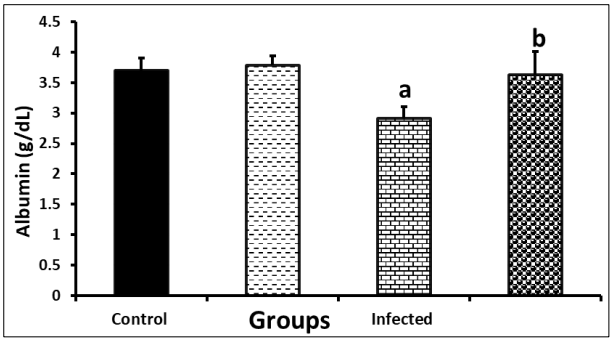

Fig 1: Effects of Mustard seed extracts on Albumin (ALB) level. Data are presented as the mean ± SE. [a] and [b] indicate significant change from control and infected group, respectively, at P < 0.05 using ANOVA followed by Duncan as a post ANOVA test.

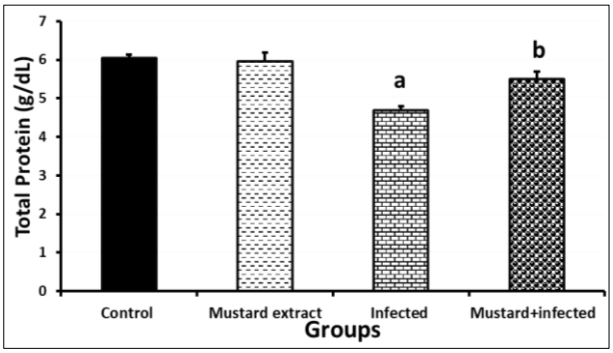

Fig 2: Effects of Mustard seed extracts on total protein level. Data are presented as the mean ± SE. [a] and [b] indicate significant change from control and infected group, respectively, at P < 0.05 using ANOVA followed by Duncan as a post ANOVA test.

Effect of Mustard seed extract on serum urea level

The levels of serum urea in infected and treated groups is graphically demonstrated in Figure (3). One-way analysis of variance was conducted to determine whether samples means are significantly different from each other. The analysis indicated that there was significant (P <0.05) increase found in blood urea of candida infected group when being compared to the control group. After 2 weeks of mustard extract treatment, the level of serum urea was significantly decreased as compared with infected group.

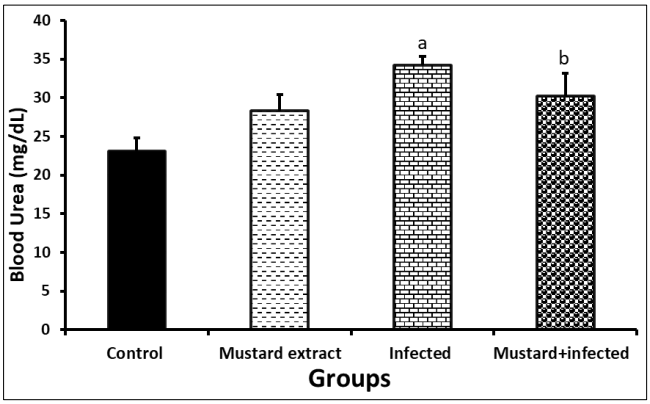

Fig 3: Effects of Mustard seed extracts on serum urea. Data are presented as the mean ± SE. [a] and [b] indicate significant change from control and infected group, respectively, at P < 0.05 using ANOVA followed by Duncan as a post ANOVA test.

Effect of Mustard seed extract on serum urea level

The levels of serum urea in infected and treated groups is graphically demonstrated in Figure (4). The analysis indicated that there was significant (P <0.05) increase found in serum creatinine of candida infected group when being compared to the control group. After 2 weeks of mustard extract treatment, the level of serum creatinine was significantly decreased as compared with infected group.

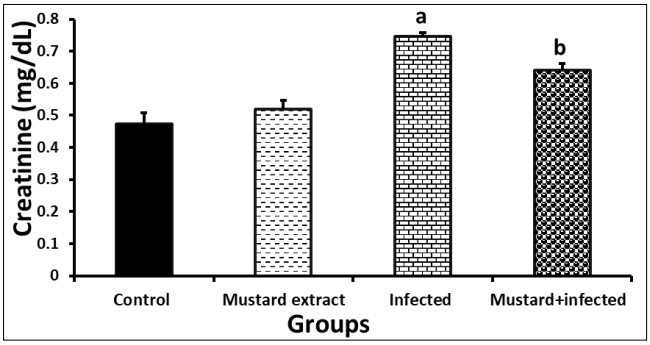

Fig 4: Effects of Mustard seed extracts on serum Creatinine. Data are presented as the mean ± SE. [a] and [b] indicate significant change from control and infected group, respectively, at P < 0.05 using ANOVA followed by Duncan as a post ANOVA test.

Histopathological investigation of kidney tissue

Alterations to the histological architecture of kidney of the experimental rats were observed. The tissue sections were analyzed as follows: Sections of kidney tissues in control group displayed preserved architecture showed normal renal architecture. Within the cortex were prominent glomeruli with distinct Bowman's capsule and cellular messengium with prominent and closely packed tubules (Figure 5A). Mustard treaded group exhibited prominent glomeruli with distinct Bowman's capsule and normal renal tubule (Figure 5B). Rats infected with Candida showed many pathological changes; apoptosis of glomeruli (Figure 5D) and degenerative real tubules was observes as well as hemorrhage between renal kidney tubules (Figure 5D). Most of cuboidal cells of renal tubules displayed cloudy swelling besides edematous exudate between renal tubules (Figure 5E). Infected group treated with the tested extract retain the normal architecture of renal tissue as shown in Figure (5F).

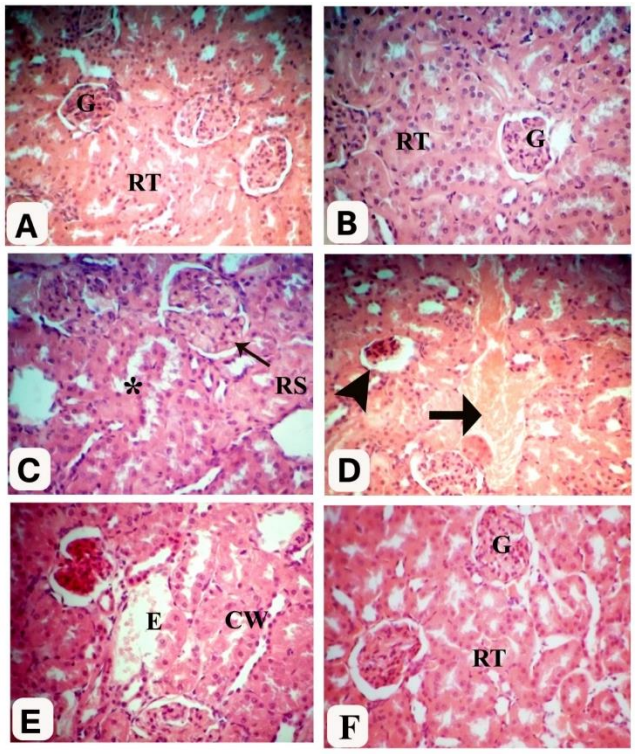

Fig 5: Histopathological findings in rat kidney: (A) control group; displayed normal morphology of kidney tissue; (B) *Mustard seed extract* -treated rats showed normal structure of renal tissue. (C) *Candidia* infected rats narrowing in renal space (RS) and degenerated renal tubules (astriks) (D) *Candidia* infected rats revealed sever hemorrhage between renal tubules (arrow besides atrophied glomerulus (arrow head) (E) *Candidia* infected rats showed edema (E) and cloudy swelling in cuboidal cells lined renal tubules (CW). (F) rats infected with *Candidia and* treated with 500 mg/kg *Mustard* seeds extract had regained its normal shape of kidney tissue. (G) glomerulus; (RT) renal tubules. Magnification (X200).

Discussion

Fungal disease specific to the urinary tract can be caused by only a few species. The most commonly implicated is Candida albicans, which accounts for more than half the cases of fungal urinary tract infections (Friedenberg, 1981). Fungal affections of the kidney develop as opportunistic infections mainly in the setting of altered host resistance resulting from a plethora of diverse causes (Morris *et al*, 2002).

Concerning level of serum total protein and albumin, mustard seed extract restore the reduction in blood total protein caused by Candida infection which was also reported with Kuhn *et al*.

(2004), accompanied by a parallel elevation in level of albumin when compared with the positive control (infected) group. On the other hand, infected rat treated with the extract didn't show any significant difference compared with infected untreated rat.

Mustard seed extract had an effect on urea and creatinine in infected rat. Candida infection caused a significant increased which had been relived after the administration of mustard seed extract. Our results is in agreement with Swenholt (2015) [17] who reported an elevation in either urea and creatinine after fungal infection.

Histopathological investigation establish causal relationships between candida infection and various histological alterations. These findings were in agreement with Chen *et al*., (2012) [4] who declared that experimentally induced candida infection in mice revealed acute renal failure characterized by glomerular and tubular degeneration.

Treatment of rats with Mustard extract largely prevented candida infection from inducing histopathological alterations in the kidney tissue as indicated by retain normal architecture of renal tissue. These results strongly supports that Mustard have powerful antioxidant and antifungal properties. The free radicals scavenging effects of these spices could be attributed to its higher polyphenols and flavones contents. Polyphenols are the most significant compounds for the antioxidant properties of plant raw materials (Rice-Evans *et al*., 1997) [16]. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators (Carocho and Ferreira, 2013) [3]. The presence of these antioxidant compounds mustard seed extract could have been the cause for the observed protection of renal tissue from experimental candidia infection.

Thus, in conclusion, the present study confirmed that mustard has a powerful antioxidant effects against candida infection induced renal toxicity. treatment with mustard seed extract was able to reduce histopathological changes as well as blood renal biomarkers.

References

1. Amarowicz R, Wanasundara UN, Karamać M, Shahidi F. Antioxidant activity of ethanolic extract of mustard seed. Food/Nahrung. 1996; 40(5):261-263.
2. Bartels H, Bohnzer M, Heierli C. Clinical chemistry. Acta, 1972; 37:193.
3. Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and chemical toxicology, 2013; 51:15-25.
4. Chen YV, Rosli R, Fong SH, Sidik SM, Pei CP. Histopathological characteristics of experimental Candida tropicalis induced acute systemic candidiasis in BALB/c Mice. Int J Zool Res, 2012; 1:12-22.
5. Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrhoeal activity of Calotropis gigantea R. Br. in experimental animals. J Pharm Pharm Sci. 2004; 7(1):70-5.
6. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica chimica acta. 1971; 31(1):87-96.
7. Friedenberg RM. Radiographic atlas of the genitourinary system (Vol. 2). Lippincott, 1981.
8. Kim HS. Do not put too much value on conventional medicines. Journal of ethnopharmacology. 2005; 100(1-2):37-39.
9. Kuhn DM, Mukherjee PK, Clark TA, Pujol C, Chandra J, Hajjeh RA, *et al*. Candida parapsilosis characterization in an outbreak setting. Emerging infectious diseases. 2004; 10(6):1074.
10. Kullberg BJ, Arendrup MC. Invasive candidiasis. New England Journal of Medicine. 2015; 373(15):1445-1456.
11. Langenberg C, Bagshaw SM, May CN, Bellomo R. The histopathology of septic acute kidney injury: a systematic review. Critical care. 2008 12(2):R38.
12. Morris BS, Chudgar PD, Manejwala O. Primary renal candidiasis: fungal mycetomas in the kidney. Australasian radiology. 2002; 46(1):57-59.
13. Myoken Y, Kyo T, Fujihara M, Sugata T, Mikami Y. Clinical significance of breakthrough fungemia caused by azole-resistant Candida tropicalis in patients with hematologic malignancies. Haematologica. 2004; 89(3):378-380.
14. Olivas-Escárcega V, Ruiz-Rodríguez MDS, Fonseca-Leal MDP, Santos-Díaz MÁ, Gordillo-Moscoso A, Hernández-Sierra JF, *et al*. & Prevalence of oral candidiasis in chronic renal failure and renal transplant pediatric patients. Journal of Clinical Pediatric Dentistry. 2008; 32(4):313-317.
15. Pappas PG, Rex JH, Sobel JD, Filler SG, Dismukes WE, Walsh TJ, *et al*. Guidelines for treatment of candidiasis. Clinical infectious diseases. 2004; 38(2):161-189.
16. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in plant science. 1997; 2(4):152-159.
17. Swenholt KC. U.S. Patent Application, 2015. No. 13/573,545.
18. Vassault A, Grafmeyer D, Naudin J, Dumont G, Bailly M, Henny J, *et al*. Protocole de validation de techues (document b,stade 3) Ann. Biol. Clin, 1986; 44:686.
19. Weichselbaum CT. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. American journal of clinical pathology. 1946; 16(3_ts):40-49.