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The effect of iron dextran injection on oral candidiasis symptoms in experimental iron deficiency anemia of laboratory rats

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Abstract. Iron deficiency anemia (IDA) is the most common type of anemia that causes various health problems and is commonly companied by oral symptoms, including oral thrush from *Candida* infection. The study assessed the role of iron status in the pathogenicity of oral candidiasis in an animal model. IDA in rats was produced by feeding on iron-free diet (five weeks), followed by inducing oral candidiasis by *Candida albican*s suspension. After the infection, animal subgroups were treated by intramuscular injection (IM) of iron dextran (ID) at 2 and 4 mg/kg once a week for three weeks and normal saline injection for comparison. Blood parameters test and tongue histopathological study were conducted. The IDA parameters and the oral thrush lesions were detected in experimental rats. IM of 2 mg ID diminished oral white patches and improved blood hemoglobin (14.533 g/dl), serum iron (109.177 µg/dl), and serum ferritin (5.276 ng/ml) and decreased total iron-binding capacity (377.000 µg/dl). Tongue sections showed normal tongue papillae, reduced inflammation and regular keratin deposition on papillae. At a 4 mg dose, despite the improvement in the blood parameters, a mild reduction was found in tongue thrush by less normal appearance of tongue papillae sections, mild inflammatory cells and hyperplasia of squamous epithelium. The study findings indicate that iron status plays a critical role in the treatment of oral thrush infection.

Keywords: haematological parameters, iron deficiency anemia, iron therapy, oral candidiasis, tongue sections

INTRODUCTION

Iron is one of the trace elements in the human body, which has important functions in cell growth and differentiation, oxygen transport, DNA synthesis, and electron transport and is a cofactor in many enzymatic reactions, including cell-mediated immune responses (Aisen *et al.,* 2001; Van & Steenkamp, 2011). Iron deficiency anemia (IDA) is a global health problem among young children and women (Agarwal & Goel, 2008). The major cases include nutritional deficiency, chronic blood loss, and reduced iron absorption (WHO, 2001), which lead to inadequate iron levels, causing many health complications (Umbreit, 2005). IDA was first clinically noted in the oral cavity of patients due to high sensitivity of oral mucous membrane to nutrients including iron to maintain its integrity (Lu & Wu, 2004). The oral manifestations involve dysphagia, glossodynia, and oral thrush (Pierro *et al.,* 2004), glossitis, angular cheilitis, dysgeusia, recurrent aphthous ulcers (Lopez *et al.,* 2016; Bhattacharya & Misra, 2017). Iron is a fundamental requirement for both the host and its pathogenic agents as an essential factor in pathogenicity (Fourie *et al.,* 2018). *Candida* spp. are the most common yeast species in the oral and vaginal mucosa, especially *Candida albicans*, which has colonized approximately one-half of the

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population of healthy individuals and has an opportunistic feature that causes infection via specific virulence factors (Hebecker *et al.,* 2014; Höfs *et al.,* 2016). Oral thrush results from *Candida* infection and appears as a discrete white lesion on the tongue, buccal mucosa, and gum lining, which is known as oral candidiasis (Takakura *et al.,* 2003). The interaction between the host immune system and the fungal virulence factors exhibits infections occurrence (Hebecker *et al.,* 2014; Höfs *et al.,* 2016). The depressed lymphocyte function in IDA patients is represented in subjects with and without oral lesions. Therefore, the growth of *Candida* in the oral cavity may be affected by other factors such as iron status in the oral epithelium, which changes its metabolism and function involving reduced thickness of epithelial compartment and diminished iron-dependent enzymes in the buccal epithelia of IDA patients (Naderi *et al.,* 2013; Wu *et al.,* 2014; Lu, 2016). Many studies used animal models, including rabbits, rats, and mice, to induce oral candidiasis (Walsh *et al.,* 2000; Kamai *et al.,* 2001; Martinez *et al.,* 2001) and assess host–*Candida* interactions during infection, providing information about oral clinical symptoms, histological changes in oral tissue, and stages of oral thrush infection (Takakura *et al.,* 2003; Hisajima *et al.,* 2008; Ibrahim & Hafez, 2012). In view of the importance of iron in physiological and immunological functions, the current study attempts to achieve the concept that raising the serum iron level to limits compatible with body's requirements is effective tool in combating oral candidiasis effects of iron deficiency. Due to limited studies on the relationship between iron level and the incidence of oral candidiasis, this study aimed to assess the effect of iron therapy on *Candida* infection and its symptoms in rat oral cavity, anemia status and determine the histopathological changes in tongue tissue.

MATERIALS AND METHODS

Animals

Male albino Wistar rats (190–210 g body weight, three months old, n=52) were used in the study. The ethical standards protocol of animal care and experiments was followed according to the animal ethical committee of Biology department- College of Science (n. 329). The animals were provided with free access to food and water in constant environment (12:12 h light: dark cycle, 25–30°C) in standard cages.

Inducing IDA in experimental rats

The rats were divided into IDA rats group (n=36) and control group (n=16). IDA was induced by feeding rats with an iron-free diet for five weeks (Kamei *et al.,* 2010), whereas the control group was fed with standard diet. The standard diet contained the following nutritional requirements: (g/kg): 200 g protein (casein), 630 g carbohydrates (sucrose, starch), 70 g fat (soybean oil), 50 g Avicel PH101(cellulose powder), 10 g vitamin mixtures, 35 g mineral mixtures, choline bitartrate 2.5 and 0.212 g ferric citrate. In the iron-free diet, the same ingredients were used except for ferric citrate. After five weeks of feeding, six rats from each IDA and control groups were sacrificed after being anesthetized with pentobarbital sodium (60 mg/kg body weight), blood samples were collected by using a cardiac puncture for haematological and biochemical measurements.

C. albicans

C. albicans strain M61B was isolated from a patient with oral candidiasis. The clinical strain was sub-cultured onto Sabouraud dextrose agar medium SDA (HiMedia, India) at 37°C for 24 h. On the day of rat treatment (tongue inoculation), the cells of *C. albicans* were harvested, washed thrice, centrifuged at 3000 rpm for 10 min, and suspended in sterile normal saline to obtain 6×10^6 cells/ml by using the McFarland technique according to the method of Collee *et al.,* (1996).

Induction of oral candidiasis in iron-deficient rats

The group of IDA rats (n=30) were treated to develop oral candidiasis infection as following: to initiate the infection, all rats were immunosuppressed by prednisolone (Bioner-Iraq, subcutaneous injection, 100 mg/kg body weight) in one day prior and after three days of cell suspension treatment (Takakura *et al.,* 2003). Also, the rats received in their drinking water a tetracycline chloride (Samara, Iraq: 0.5 mg/ml) before the day of fungal induction and during the experimental period (Takakura *et al.,* 2003,

Junqueira *et al.,* 2009). On the day of suspension treatment, all rats were anesthetized via intramuscular injection with 0.1 ml of 1:2 ketamine and xylazine (Turner & Albassam, 2005). 0.2 ml of *C. albicans* strain M61B suspension (6×10^6 cells/ml) is dropped by syringe in rat's oral cavity and spread on tongue surface by a small cotton pad that soaked in the cell suspension. All rats were left without drinking water for three hours. This procedure was repeated on all rats for three continuous days (Junqueira *et al.,* 2009). After a week of inducing infection, the rats' oral cavity was examined for tongue thrush after anesthetizing animals. The thrush symptom was detected by a white lesion on the tongue surface. Oral swabs from each rat were obtained and cultured on SDA medium.

Effect of iron injection

After two weeks of detection oral candidiasis symptoms in rats, the IDA rats with oral candidiasis were divided randomly into three groups (each ten rats): group A: intramuscular injection (thigh muscle: biceps femoris) with 2 mg/kg body weight of iron dextran (ID, 50 mg/ml injection solution: ferric hydroxide and dextran liquid complex-Syria); group B: intramuscular injection with 4 mg/kg body weight of ID; and group C: intramuscular injection with normal saline (positive control). The injection is once a week for three weeks. The rats were free access to iron-free diet. Group D: healthy rats without anaemia and without oral thrush (negative control, n=10). Animals were monitored for tongue thrush symptoms, and oral swabs were obtained from all groups weekly and cultured to detect *Candida* growth. At the end of the injection period, six rats from each group were sacrificed for blood test, and tongue samples were obtained for histopathological study.

Haematological and biochemical analysis

Complete blood count was obtained by using an automated blood count system (SYSMEX XT 2000i blood analyzer) including RBC (red blood cells), Hb (haemoglobin), Hct (haematocrit), MCV (mean cell volume), MCH (mean cell haemoglobin), MCHC (mean cell haemoglobin concentration) and RDW (red cell distribution width). Serum iron, total iron- binding capacity (TIBC), zinc, and magnesium were measured using commercial kits from Biolabo (France) and Randex (England), serum ferritin by Elisa kit (CALBIOTECH Cat. No. FR248T). Transferrin saturation (TS) was calculated using the following equation: TS (%) = 100 × (serum iron/TIBC) (Ceriotti & Ceriotti, 1980).

Histological study

The rat tongue samples were fixed in 10% neutral formalin solution following the standard protocol for histological preparation (Lunea, 1968). Sections with 7 μm thickness were obtained from embedded blocks and stained with periodic acid Schiff (PAS) and counterstained with Mayer's haematoxylin for fungal detection (Steinke *et al.,* 2018).

Statistical analysis

The data of two groups were analyzed by t-test, the data of many groups were analysed by one-way ANOVA using SPSS version 21. The comparisons between means were made using revised least significant differences. Differences were considered to be significant at $p\leq0.05$. Data are presented as means ± S.D. The yeast growth was analyzed by Chi-square test.

RESULTS

Haematological and biochemical parameters in IDA rats

The IDA rats displayed a significant difference in haematological parameters compared with the control group that was fed with standard laboratory diet (Table 1). The IDA group showed significant ($p\leq0.05$) reduction in red blood cell (RBC) counts, haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) values compared with the control group. There was a significant difference in white blood cell counts between the two groups. The statistical analysis showed significant ($p\leq0.05$) decrease in the biochemical parameters including serum iron, ferritin, Transferrin saturation (TS), and zinc concentrations in rats fed with iron-free diet for five weeks compared with the control group (Table 1). The total iron- binding capacity (TIBC)

values were significantly elevated in the IDA group. No significant change in magnesium level was observed between the tested groups.

Oral candidiasis symptoms in IDA rats

The symptoms of oral candidiasis were macroscopically observed after one week of *C. albicans* inoculation, which was characterized by white lesions on the tongue surface that showed positive growth on SDA medium compared to the control group with normal tongue appearance and negative growth results.

Effect of ID injection on IDA rats with oral thrush

Oral symptoms and Candia growth

2 mg ID injection reduced tongue symptoms after two weeks of treatment. These symptoms disappeared completely at three weeks post-injection (Figure 1a). Treatment with a dose of 4 mg displayed a smaller decrease in oral candidiasis at two and three weeks post-injection (Figure 1b) compared with the normal saline injected group, which exhibited clear white thrush lesion (Figure 1c). The growth of *C. albicans* taken from iron-treated groups displayed variation in culture according to the iron dose (Table 2). The oral swabs from rats treated with a dose of 2 mg ID showed a negative growth throughout the three weeks of treatment as the health group, whereas those treated with a dose of 4 mg ID showed a positive growth as the normal saline injected group.

Hematological and biochemical parameters

ID injection in IDA rats with oral candidiasis (groups A, B) improved hematological parameters at three weeks post-injection (Table 3). A significant ($p \leq 0.05$) increase was found in Hb, Hct, MCV, and MCH levels compared with the normal saline injected group (C). In comparison with the healthy rats group (D), the iron injected groups displayed no significant differences in MCH and RDW values. No significant difference was found in MCHC level among all groups. The group A showed significant increasing in WBC levels compared to all groups. The biochemical parameters, including serum iron, ferritin, TS, zinc, and magnesium, in iron injected groups (A, B) were significantly increased ($p \leq 0.05$) at three weeks post-injection (Table 3). An ID dose of 4 mg (group B) showed no significant differences in TIBC value, which was elevated in rats injected with normal saline. At 2 mg ID dose (group A), the results revealed no significant differences in serum iron, TIBC, TS, Zn, and Mg were observed compared with the healthy rats group (D), while it differs significantly in ferritin level.

Table 1. The hematological and biochemical parameters in experimental rats' groups.

Parameters	IDA	Control
RBC(µl)	$6.686 \times 10^6 \pm 0.162^b$	$8.237 \times 10^6 \pm 0.312^a$
Hb(g/dl)	9.600 ± 0.655^b	16.720 ± 0.360^a
Hct (%)	30.100 ± 1.650^b	46.500 ± 1.509^a
MCV(fl)	45.091 ± 1.665^b	56.452 ± 0.472^a
MCH (pg/cell)	14.358 ± 0.709^b	20.298 ± 0.635^a
MCHC (g/dl)	31.893 ± 1.345^b	35.956 ± 0.808^a
RDW (%)	27.910 ± 1.803^b	21.133 ± 1.096^a
WBC (µl)	$7.400 \times 10^3 \pm 1.113^b$	$10.120 \times 10^3 \pm 0.623^a$
Fe (µg/dl)	37.233 ± 1.435^b	96.833 ± 0.230^a
TIBC(µg/dl)	454.755 ± 11.554^a	328.833 ± 6.932^b
TS (%)	13.292 ± 0.843^b	29.105 ± 0.567^a
Ferritin (ng/mL)	3.920 ± 0.183^b	18.073 ± 0.756^a
Zn (µg/dl)	33.366 ± 1.001^b	38.033 ± 1.461^a
Mg (mg/dl)	3.100 ± 0.100	$3.386 \pm 0.161^{n.s.}$

IDA: iron deficient group. Mean±standard deviation. Small different letters referred to significant at $p \leq 0.05$. fl: femtoliter. pg: picogram. n.s.: not significant.

Figure 1. Tongue thrush symptoms in experimental groups: a, b, c after three weeks of injection. a: 2 mg ID injection, b: 4mg ID injection, c: normal saline injection, d: healthy rat. Oral thrush (➞).

Table 2. Growth of *C. albicans* from oral swabs of the treated groups.

Growth onto SDA medium	Group A	Group B	Group C	Group D	p≤0.05
1 week	+	+	+	-	0.007
2 week	+	+	+	-	0.180
3 week	– (8) + (2)	+	+	-	0.030

+ positive growth, _ negative growth

Table 3. The hematological and biochemical parameters in the experimental rats' groups after 3 weeks of iron-dextran injection.

Parameters	Group A 2 mg/kg injection	Group B 4 mg/kg injection	Group C Normal saline injection	Group D Healthy rats
RBC(µl)	$8.920\times10^{6}\pm0.261^{a}$	$7.066\times10^{6}\pm0.699$ b	$6.603\times10^{6}\pm0.456^{b}$	$8.450\times10^{6}\pm0.312^{a}$
Hb(g/dl)	14.533 ± 0.923^{b}	12.700 ± 0.435^{c}	9.300 ± 0.900^{d}	16.000 ± 0.360^{a}
Hct(%)	43.166 ± 0.251^{a}	39.233 ± 1.379^{b}	28.266 ± 4.194^{c}	46.000 ± 1.509^{a}
MCV(fl)	49.366 ± 1.209^{b}	53.100 ± 3.119^{a}	42.807 ± 3.012^{c}	55.766 ± 0.472^{a}
MCH(pg/cell)	16.533 ± 1.234^{a}	17.800 ± 1.417^{a}	14.080 ± 1.985^{b}	18.033 ± 0.635^{a}
MCHC(g/dl)	33.660 ± 0.152	32.370 ± 1.193	32.901 ± 1.833	$34.780\pm0.808^{n.s.}$
RDW(%)	21.433 ± 1.569^{b}	20.233 ± 1.201^{b}	29.972 ± 2.128^{a}	21.800 ± 2.251^{b}
WBC (µl)	$17.126\times10^{3}\pm2.583^{a}$	$11.786\times10^{3}\pm1.278^{c}$	$10.693\times10^{3}\pm0.344^{c}$	$15.453\times10^{3}\pm1.001^{b}$
Fe (µg/dl)	109.177 ± 6.811^{b}	125.733 ± 4.168^{a}	32.233 ± 4.605^{c}	105.833 ± 11.164^{b}
TIBC (µg/dl)	377.000 ± 45.116^{b}	441.611 ± 24.759^{a}	535.648 ± 46.545^{a}	342.166 ± 40.250^{b}
Ts (%)	29.290 ± 3.008^{a}	28.564 ± 1.965^{a}	10.714 ± 0.394^{b}	31.786 ± 5.595^{a}
Ferritin(ng/mL)	12.276 ± 0.612^{b}	8.166 ± 0.552^{c}	3.373 ± 0.902^{d}	17.073 ± 0.756^{a}
Zn (µg/dl)	40.666 ± 1.154^{a}	37.333 ± 0.577^{b}	30.000 ± 1.000^{c}	42.000 ± 1.000^{a}
Mg (mg/dl)	3.066 ± 0.111^{a}	3.330 ± 0.204^{a}	2.213 ± 0.275^{b}	3.386 ± 0.161^{a}

Mean±standard deviation. Small different letters referred to significant at $p\leq0.05$, n.s.: not significant.

Histopathological results

Tongue sections of control rats (healthy without IDA and oral candidiasis) showed the normal structure of tongue tissue. Tongue dorsal surface sections showed in anterior part simple conic papillae (Figure 2a). The fungiform papillae (less in number) being short and broad with rounded base and containing taste buds (Figure 2a). The posterior part showed filiform papillae (Figure 2b). The striated muscular tissues appeared surrounded by keratinized stratified squamous epithelial layer (mucous membrane). The bundles of muscle fibers are separated by connective tissue. The same sections of normal rat showed the conjunctive tissues parts, which appeared beneath the epithelium with high vascularity (rich blood vessels) and muscle fibers.

The tongue dorsal sections of group IDA rats with oral candidiasis showed variable changes. The anterior part of tongue displayed atrophied conic and fungiform papillae with hyperkeratosis (Figure 3a, b). *Candida albicans* cells showed fusion with the keratin layer and with the desquamated layer of papillae (Figure 3c). A thick layer of squamous cells was observed. Loosing of irregular connective tissue with irregular striated muscle. The posterior dorsal part contains multiple tissue lesions which showed epithelial hyperplasia, loss of filiform papillae and hyperkeratosis (Figure 3d and 3e). Observed presence of inflammatory infiltrate in the lamina propria also disorganization of basal layer.

The IDA with oral thrush which were injected with 2 mg ID (group A) showed enhancement in tongue histological features and showed recovery in most tissues. The anterior dorsal surface of tongue section showed regenerated conic papillae (Figure 4a and 4b). Observed normal squamous epithelial layer and regular basal layer. Also normal striated muscle fibers and connective tissue were found. The posterior part of tongue section (Figure 4c and 4d) revealed a number of regenerated filiform papillae which some with pointed endings. The squamous epithelial layer showed regular keratin deposition. The lamina propria is normal. There are mild inflammatory cells. The muscles strands are separated with normal connective tissue.

The results showed that 4 mg iron dosage (group B) showed less effect. The anterior dorsal surface section revealed lower number of simple conic papillae and hyperplasia of squamous epithelium (Figure 5a and 5b). The striated muscles were arranged regularly and separated with collagen fibers. Branches of secretory ducts related to mucous and serous glands were also observed. The observations in the posterior part of tongue section showed regeneration of filiform and fungiform papillae with less keratinized layer and pointed endings (Figure 5c and 5d). Also

observed presence of mild inflammatory cells in lamina propria. The muscles were distributed regularly, and the mucosa was attached well under the connective tissue.

The histological observations on the tongue of IDA rats with oral candidiasis injected with normal saline (group C) showed the infection. The anterior dorsal surface section showed atrophied conic papillae with large layer of squamous epithelium (Figure 6a). The section showed that *C. albicans* cells were invaded the tissue and stained purple with PAS (Figure 6b). The posterior dorsal part contain multiple tissue lesions showed epithelial hyperplasia, loss of filiform papillae and hyperkeratosis (Figure 3c and 3d). The section showed the hyper-proliferation of squamous epithelium, also the presence of inflammatory infiltrate in the lamina propria.

DISCUSSION

The study results showed that feeding rats with iron-free diet for five weeks reduced Hb, serum iron, and ferritin levels, which agree well with other studies (Fernandes *et al.,* 1997; Wayhs *et al.,* 2004). Feeding rats with iron-free diet for several weeks affected the iron status in the body that was reflected by the reduction in Hb levels (Linberg *et al.,* 1998). IDA can be induced by dietary method through food with reduced iron absorption (Lin *et al.,* 2003). The current results showed that the low level of MCH, MCHC, and HcT was related to the deficiency in iron level, which was reflected in diminished RBC formation and its Hb content. The reduced Hb levels were related to defective cell division process and low rate of Hb production, leading to microcyte formation. The low value of HcT resulted from increased plasma volume compared with the low volume of microcytic RBC (Lambert & Beris, 2009). IDA rats had low level of ferritin and TS and high level of TIBC, which can be explained by the reduction in ferritin concentration that is related to the depletion of stored iron (Wang & Pantopoulos, 2011). This depletion impairs iron delivery to the plasma that reduces iron transport protein transferrin, which is one of the markers of IDA (Bermejo & Garcia-Lopez, 2009). The depression in iron storage increases the ability of the plasma to bind iron with transferrin protein that is expressed as TIBC (Naigamwalla *et al.,* 2012). To meet the body's physiological demands, the body produces immature RBC in the blood stream with inadequate Hb content, causing variation in RBC volume that increases RDW (Cesana *et al.,* 1991), which is represented in the study results. The study results showed that the iron-free diet displayed low serum zinc level in feeding rats that may related to increased zinc- protoporphyrin in hemoglobin synthesis instead of heme when iron levels decreased in the body (Sandstead *et al.,* 2008).

The inoculation of *C. albicans* (6×10^6 cells/ml) in rat oral cavity resulted in growing colonies. The macroscopic observation displayed the local symptoms of oral candidiasis characterized by lesions as white patches on the tongue surface. In this study, the use of tetracycline in drinking water raised *C. albicans* colonization in the rat oral cavity that is related to the interruption of the normal murine microbiota growth in the oral cavity, which facilitated *Candida* growth, in addition, rubbing the cotton swab would scratch the tongue surface and promote *Candida* to invading tissue (Takakura *et al.,* 2003). The invading process of *C. albicans* in rat tongue may be explained by the secretion of lipase and other enzymes that destroyed the host epithelium through the desmosomal Bridge and allowing the penetrated of the intracellular space (Reichart *et al.,* 1994). The extracellular secretion of aspartyl proteinases 1-9, phospholipase A, and lysophospholipase destroyed the protein substrate (Samaranayake *et al.,* 1994). During invading, *Candida* cells formed clusters, which attached to the mucoidal substance of tongue surface and then formed hyphae. The hyphae penetrated the tongue surface within 3 h after inoculation; the covered material plays a role between *Candida* and tongue mucosal epithelium interaction (Hisajima *et al.,* 2008).

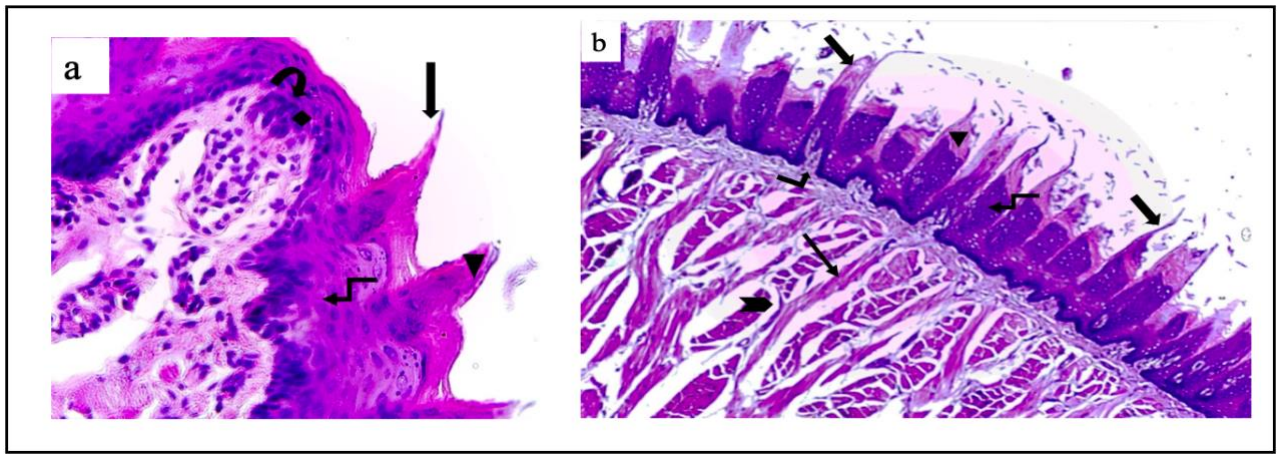

Figure 2. Dorsal surface sections of control tongue rat group. a: Simple conic papillae (⬇), b : fungi form papillae (↷), filiform papillae (↘). Stratified squamous epithelial tissue (↵), mild keratin coated the papillae (▼), groups of striated muscle (➢) separated with connective tissue (◣), lamina propria (⬏), test bud (◆). (PAS; H&E) stain (a: 40X; b: 10X).

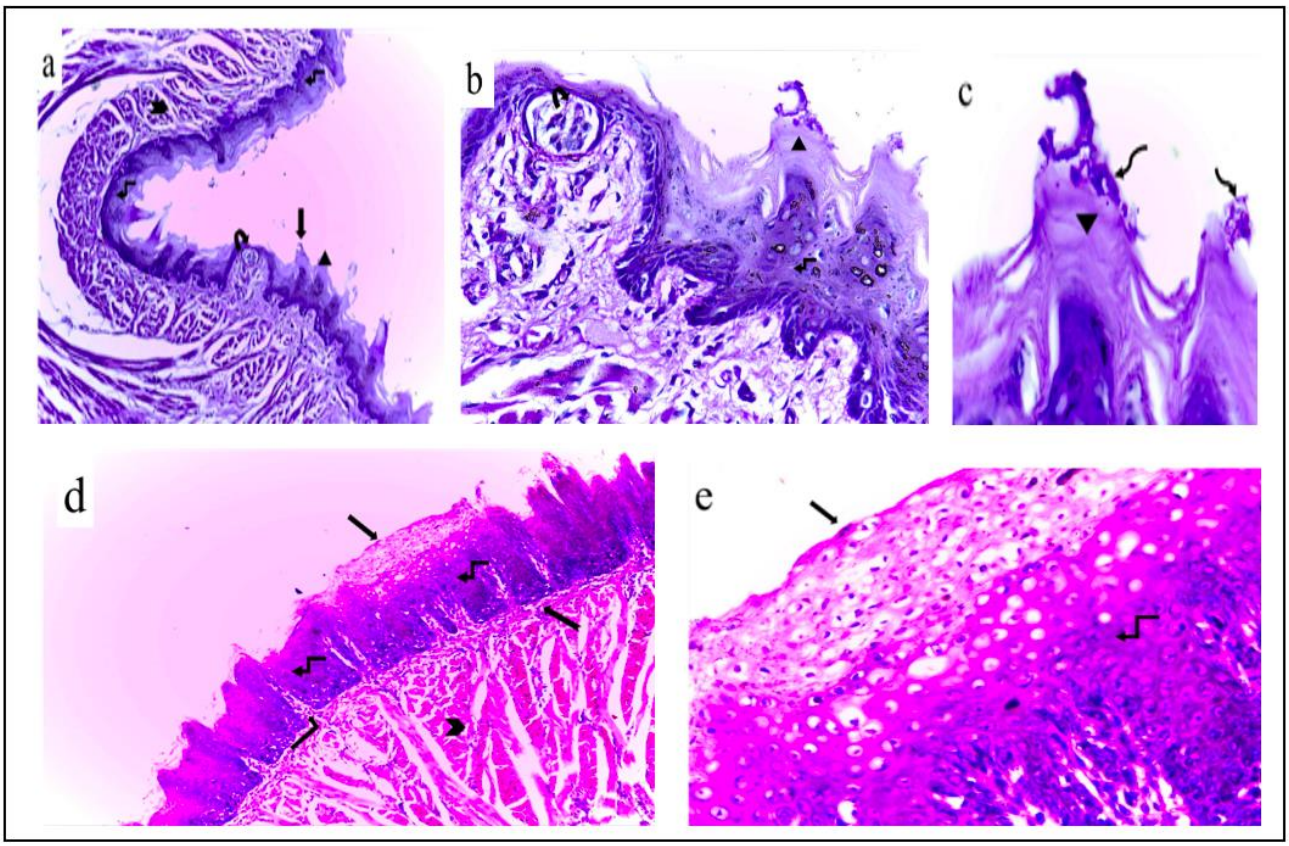

Figure 3. Dorsal surface sections of tongue in IDA rat with oral candidiasis. a: atrophied conic papillae (⬇) with hyperkeratosis (▲), b: fungiform papillae (↷), c: *C. albicans* stained to purple (↘) invading the keratin layer (▼), d, e: tissue lesion showed epithelial hyperplasia and loss of filiform papillae (⬅). Thick stratified squamous layer (↵), striated muscle fiber (➢), lamina propria showed inflammatory infiltrate (⬏), inflammatory cells (➡). (PAS; H&E) stain, (a, d: 10X, b, e: 40X, c: 100X).

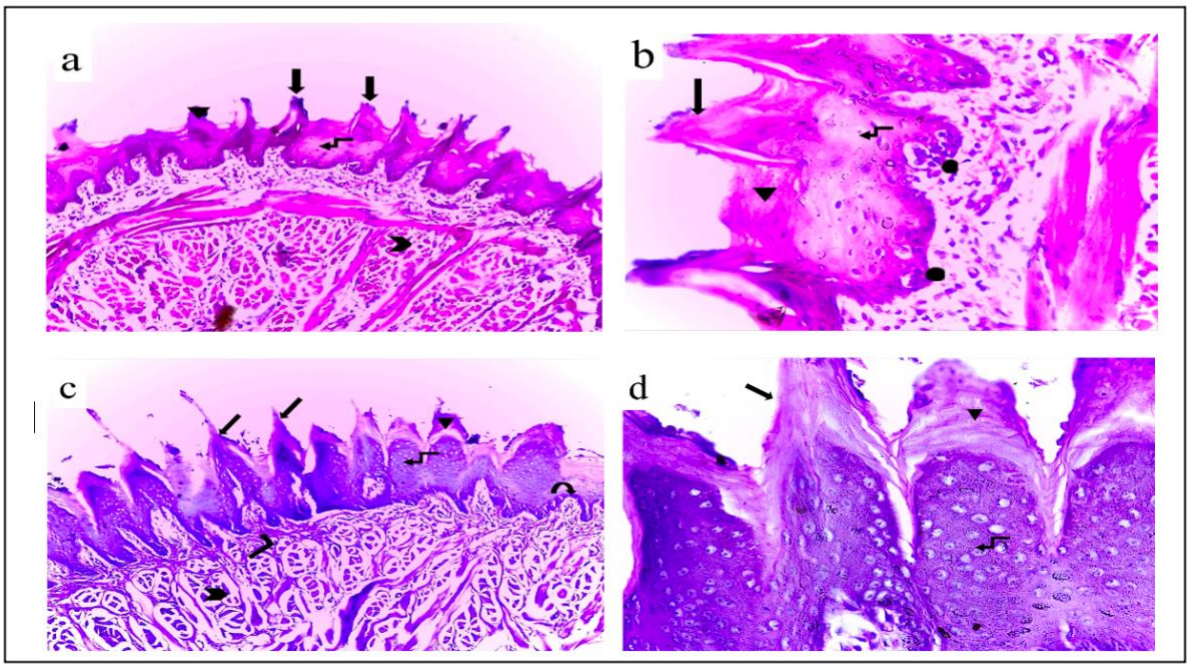

Figure 4. Dorsal surface sections in tongues of 2 mg iron dextran injected in IDA rat with oral candidiasis. a, b regenerated conic papillae (⬇), c, d: regenerated filiform papillae (⬅) and fungiform papillae (↷), keratin layer (▼), stratified squamous epithelium (↵), striated muscle fiber (➤), regular basal cells (●), normal lamina propria (⮥). (PAS; H&E) stain, (a, c: 10X, b, d: 40X).

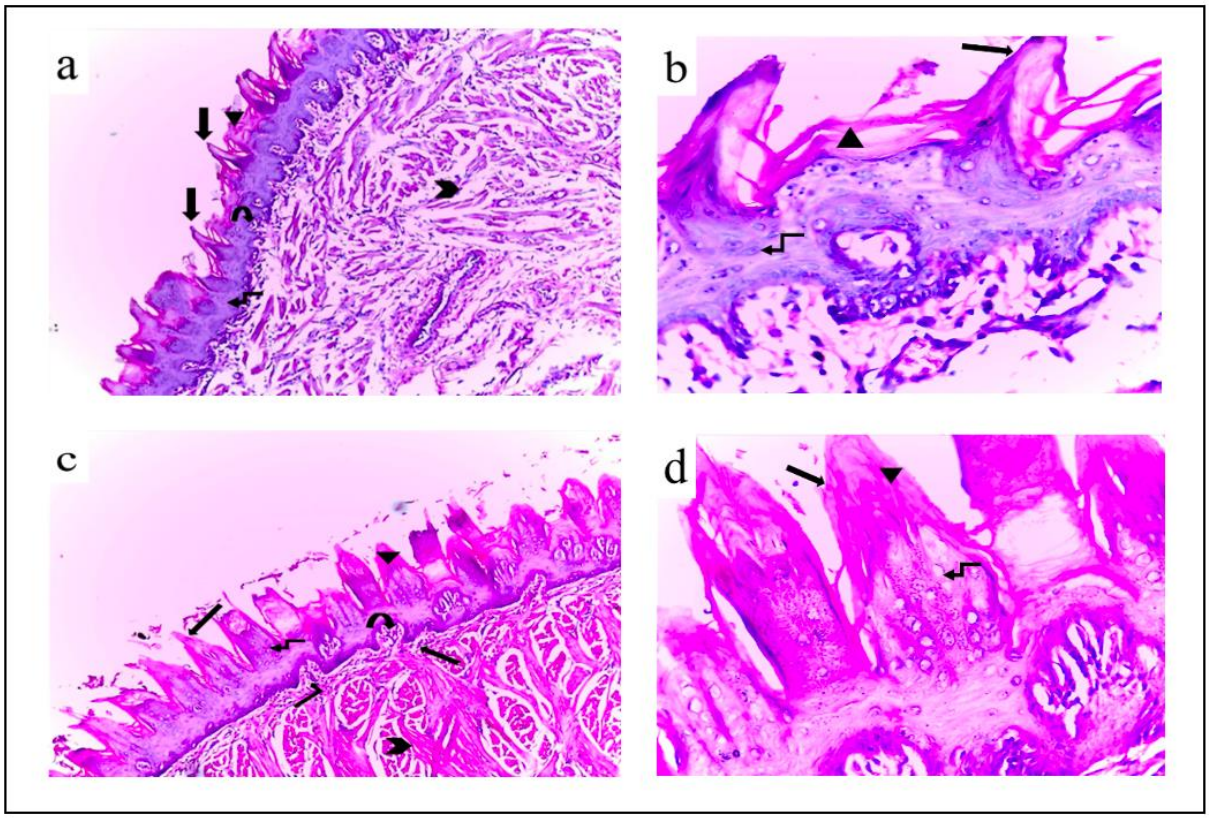

Figure 5. Dorsal surface sections in tongues of 4 mg iron dextran injection in IDA rat with oral candidiasis. a, b regenerated conic papillae with thin keratin layer (⬇), c, d regenerated filiform papillae (⬅) and fungiform papillae (↷), keratin layer (▼), stratified squamous epithelium (↵), regular striated muscle fiber (➤), mild inflammatory cells (➡) in lamina propria (⮥). (PAS; H&E) stain, (a, c: 10X, b, d: 40X).

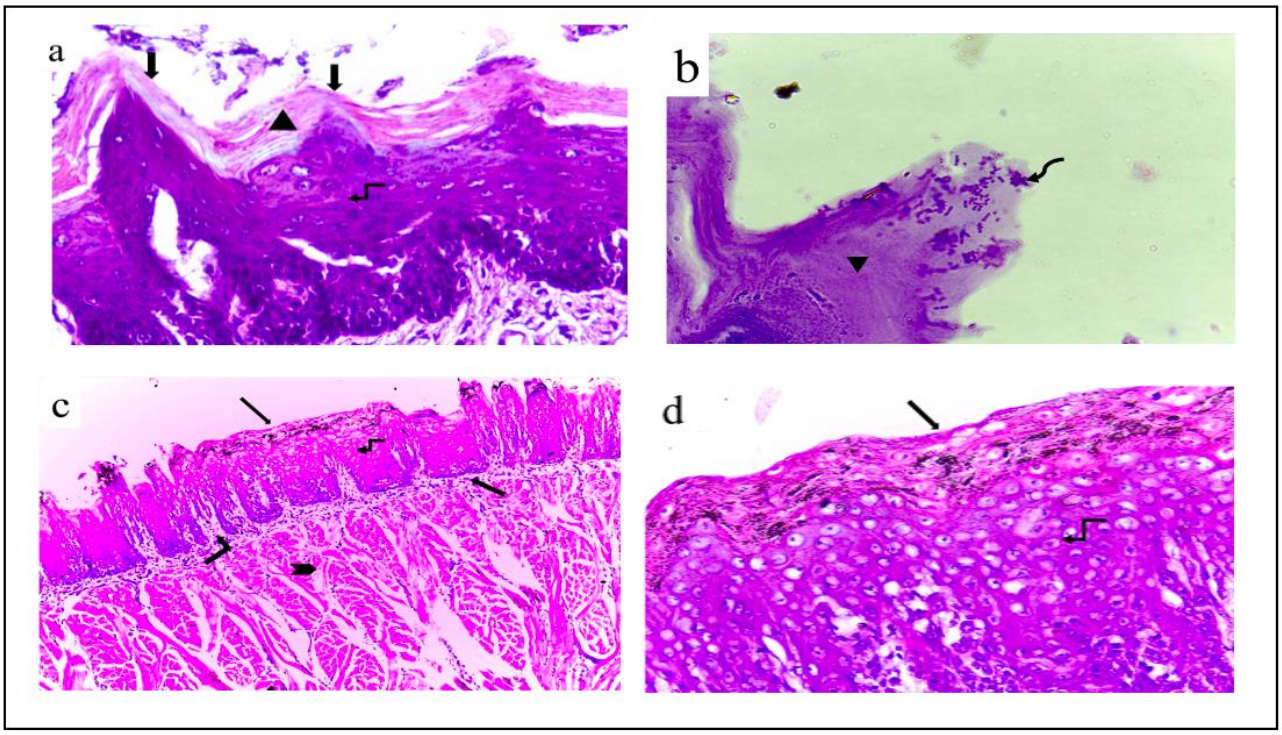

Figure 6. Dorsal surface sections in tongues of normal saline injection in IDA rat infected with oral candidiasis. Atrophied conic papillae (⬇) with hyper keratosis (▲), b: *C. albicans* stained to purple (➘) invading keratin layer, (▼), c, d: tissue lesion showed epithelial hyperplasia and loss of filiform papillae (⬅). Large layer of stratified squamous epithelium (⮢), inflammatory cells (➡), muscle fibers (➤), inflamed lamina propria (⮡). (PAS; H&E) stain, (a, d: 40X, b: 100X, c: 10X).

The tongue sections of IDA rats with oral candidiasis displayed that epithelial lining was mildly inflamed. A thick layer of large squamous cells and inflammatory cells was observed in all epithelial tissue. Spongiosis; hyperkeratosis; and large, short, and flat papillae were observed. *Candida* cells appeared embedded within the keratin and desquamated layer of papillae. All these findings were also reported by Junqueira *et al.* (2009), who revealed that the symptoms of oral candidiasis in rats were distinguished by several epithelial lesions that were histologically detected by epithelial hyperplasia, loss of filiform papillae, hyperkeratosis, spongiosis, and inflammatory infiltration. Also the study of Niyogi *et al.* (2019) showed that the pathogenesis of oral candidiasis in mice tongue sections appeared as papillary proliferation, disorganization of basal cells, loosed of papillae and hyperkeratosis. The current study showed that IDA induced by feeding rats with iron-free diet (five weeks) may cause many histological changes in the tongue that were related to many reasons explained by some investigations. The increased lipid peroxidation in cells raised mitochondrial membrane fragility (Knutson *et al.,* 2000). The up regulation in iron absorption in IDA gastrointestinal cells elevates copper absorption, which promotes the regeneration of reactive radicals that damage lipid and DNA (Walter *et al.,* 2002). The insufficient iron in oral epithelium impairs kinetics of cell division through frailty in iron-dependent enzymes of metabolism (Van Wyk & Steenkamp, 2011). The decrease in skeletal muscles facilitates the invading process of *C. albicans* in rat tongue and causes pathogenicity (Ibrahim & Hafez, 2012). The iron therapy for IDA rats with oral candidiasis via intramuscular injection revealed the enhancement in serum iron, ferritin, and TS and elevated Hb, RBC counts, MCH, MCHC, and Hct compared with the IDA parameters in normal saline injection. These results may be related to the improved iron content that is reflected by reduced TIBC as demonstrated by Kotze *et al.* (2009).

Iron treatment with a dose of 2 mg/kg body weight displayed beneficial effect compared with a dose of 4 mg/kg. These results were indicated by the improved serum iron level, increased blood parameters, and decreased oral candidiasis symptoms in rat treatment groups. Moreover, the macroscopic observations of rat tongues showed healing in the third week and negative growth of oral swabs on SDA medium. Injection with a dose of 4 mg/kg was less effect on IDA rats with oral candidiasis due to the mild reduction of oral lesions at second and third weeks post-injection and positive *Candida* growth on oral swabs. In this group, a high level of serum TIBC was found despite the elevated levels of serum iron and ferritin, which increased Hb, RBC count, and Hct. These observations indicate that a dose of 4 mg/kg is excessive, which may promote fungal infection. The defect in immunological responses, especially T-helper cells, to *C. albicans* and the increased animal susceptibility as response to overload dose were reported (Mencacci *et al.,* 1997). The high iron levels increased microbial pathogenicity via a negative effect on the antimicrobial functions of neutrophils, monocytes, and natural killer cells (Miceli *et al.,* 2006).

The study results showed that intramuscular injection of 2 mg/kg iron was effective in treating oral thrush by diminishing white patches. The histological examination displayed normal filiform papillae and regular keratin deposition that may be related to the enhancement of oral epithelium metabolism or local biochemical changes involved in the resistance to the pathogenicity of *Candida* hyphal form (Higgs & wells, 1973). Post-injection with 4 mg/kg showed changes in some features of the tongue sections by hyperkeratosis and hyperplasia of squamous epithelium, which had more severe effect than a dose of 2 mg (caused iron restoration and regeneration of epithelial tissue).

CONCLUSION

In conclusion, the use of laboratory animals as model for oral candidiasis in IDA support our results to observe the blood parameters and tongue histopathological changes during iron treatment. The intramuscular injection of ID at a dose of 2 mg/kg body weight was sufficient to diminish iron deficiency and reduce oral candidiasis symptoms during three weeks, while increasing the dose to 4 mg/kg body weight showed less effective either in oral candidiasis symptoms or tongue histopathology despite enhancing the blood parameters (serum iron, serum ferritin, and TIBC). Therefore, our results conclude that the iron therapy in iron deficiency anemia caused iron restoration and enhance the body defenses that reduced fungal growth.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest in this study.

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