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Histological effect of contraceptive pills on female mice infected with E.histolytica

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Abstract

Entamoeba histolytica is a parasitic pathogenic protozoan that causes amebiasis in humans. Evidence suggests that combined oral contraceptives (COCs) influence parasitic infection development and progression, as well as host susceptibility to infection and the resulting differences in immune response. The experiment was carried out on 50 female white mice, 10 of which were healthy (control negative) and 40 of which were infected with E.histolytica. The results were shows accumulation of lipid, dilation of sinusoid, increase inflammatory cell infiltration, slightly increase kupffer cells in liver and hyperplasia of Goblet cell with Lymphoid tissue hyperplasia in intestine .In this research shown the virulence of COC on liver and intestine of the infected mice

Keywords
COC, E.histolytica, Histological, Amebiasis.

Entamoeba histolytica is a parasitic pathogenic protozoan that causes amebiasis in humans. Infection with E. histolytica can be asymptomatic or cause severe disease such as amebic colitis and amebic liver abscess. Amebiasis is still a major cause of morbidity and mortality around the world. It is estimated that E. histolytica infection kills more than 55,000 people each year (Shirly et al.,2018). Diarrhea is the third leading cause of death in children under the age of five worldwide, with amebic colitis being the leading cause of severe diarrhoea in low-income countries (Shirly et al.,2018). Fulminant amebic colitis is a rare but potentially fatal complication; on average, more than half of people with severe colitis die (Shirly et a.,2016). E. histolytica infection is also a concern among returning travellers with infectious gastrointestinal disease, with an incidence of 14/1000 ill travelers (Swaminathan et al.,2016). Nitroimidazoles are the only treatment for invasive amebiasis. Nitroimidazoles are toxic drugs, and resistance has developed in other anaerobic protists (Shirly et al.,2018). The parasite E. histolytica comes in two stages: cyst and trophozoite. The infectious cyst is ingested from fecally contaminated food or water, or through oral-anal sexual practises, to begin the life cycle of E. histolytica. The cysts pass through the stomach and small intestine, excysting and forming invasive trophozoites in the intestine lumen. Trophozoites can enter the large intestine through the mucus layer. Colitis and liver abscess are both associated with extensive tissue damage. Amebic colitis, for example, is characterised by colonic ulcers, parasite invasion into the lamina propria, and infiltration of inflammatory cells such as neutrophils (Wu,2017).

Oral contraceptives containing oestrogen and progesterone are classified as "combined" or "uncombined," with "combined" containing both progesterone and oestrogen and

"uncombined" containing only progesterone (Benagiano et al., 2006; Fleischman et al., 2010). Progesterone is a hormone that prevents pregnancy, whereas oestrogen controls monthly bleeding, so it is primarily used to prevent pregnancy. The precise and safe application of this type of oral contraceptive demonstrates its efficacy (Sitruk-Ware et al., 2013).

Material and methods

This study included fifty females albino mice divided into five groups of ten mice each. forty mice were given (1Ч103 cell/ml) to infect and ensure the mice were infected by examining their faeces for ten days. After 3-4 days, the infection occurs. A group frome ten mice that were not infected served as the negative control. Throughout the experiment, the mice were inoculated orally with a single dose every day. Group one (non-infected): inoculated orally by stomach tube (0.1ml/day) of normal saline consider it as control negative.Group two (infected): inoculated orally by stomach tube (0.1ml/day) of normal saline consider it as control positive. Group three (infected): inoculated orally by stomach tube (0.04 ml/day) of hormonal contraceptives . Group four (infected): inoculated orally by stomach tube (0.1 ml/day) of metronidazole. Group five(infected): inoculated orally by stomach tube (0.1 ml/day) of metronidazole and (0.04 ml/day) of hormonal contraceptives.

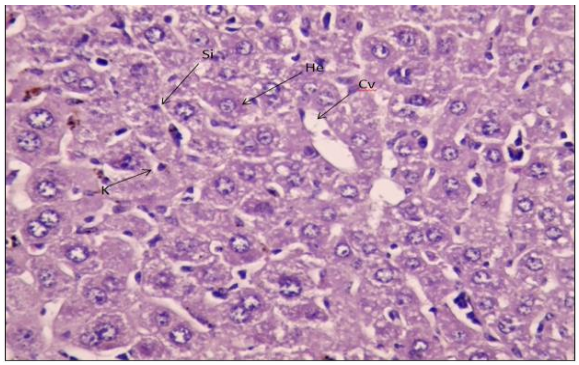
Result

Histological study of female liver

Histological changes in the liver of infected mice revealed this result in the group that was not infected with E.histolytica (control negative G1) the histological liver structure was showing the appearance of liver section with central veins surrounded by hepatocyte threads with central nuclei and sinusoids are lined by kupffer cells in the Figure (1).While the liver section of control positive (G2) showing necrosis in hepatocyte with infiltration inflammatory cells figure(2) ,although histological change in liver mice that ingested orally contraceptive pills (G3) was sever damage occurred with abundant accumulation inflammatory cells infiltration with dilation of sinusoid and lipid accumulation in figure (3). Whereas liver section of liver mice that treated with metronidazole (G4) showing still of focal area of necrosis with inflammatory cells infiltration and sinusoid dilation in figure (4)and contraceptive pills and metronidazole group(G5) showing mild deplation of Glycoproteins (Gly) and sinusoid(Si) dilation in figure (5).

Histopathological study of female intestine

The histological study intestine in mice with different group shown this result. In the group without any infection (control negativeG1) showed that the histological section of intestine was normal structure appearance of intestinal villi with normal appearance of sup mucosa gland was showing in figures (6),and in control positive (G2) that showing histological change in the intestine section shortening of intestinal villi and of hyperplasia of lymphoid tissue (LT) (peyers patch) in figures(7) . While histological change in the intestine who mice ingested orally contraceptive pills (G3) showing that histological change in large intestine section of hyperplasia of Goblet cell with shortening villi with Lmphoid tissue hyperplasia showing in figures (8). Whereas section of intestine that treated with metronidazole (G4) showing of looks like to normal appearance intestinal villi normal but still with Goblet cell hyperplasia in figures(9) and histological change in intestine where the group treated with contraceptive pills and metronidazole showing that wide of intestinal villi with hyperplasia of Goblet cell and normal appearance of lymphoid tissue (peyers patch)(10).



Figure(1) :A Section of liver of mice (G1) was showing of normal histological structure appearance which consist of central vein (CV) and surrounded by a thread hepatocytes (He) between the sinusoid (Si), and kupffer cell (10x.H&E)

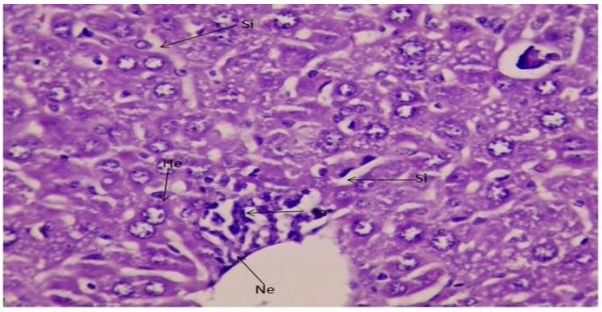


Figure (2): A Section of liver of mice (G2) which showing focal area of necrosis with inflammatory cell infiltration (In),Hepatocyte necrosis(He) and sinusoid(Si) (40x.H&E).

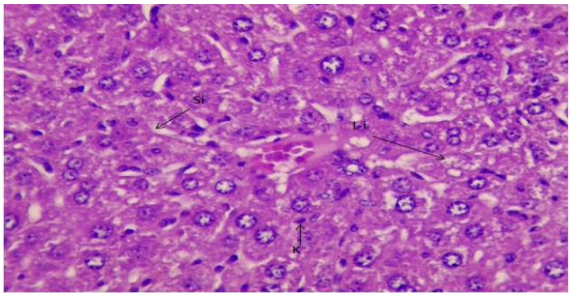
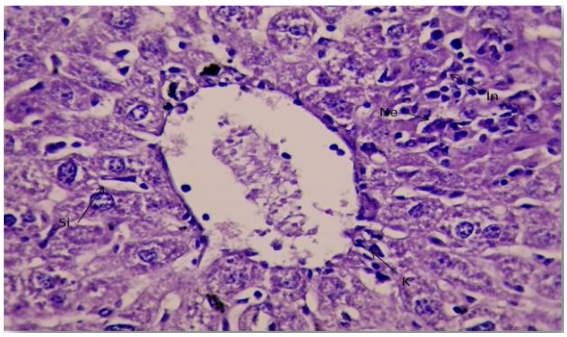


Figure (3): A Section of liver of mice in (G3) showing accumulation of lipid(Li) , dilation of sinusoid (Si), increase inflammatory cell (In) infiltration, slightly increase kupffer cells (10x.H&E).



Figure(4) : A Section of liver of mice that treatment with metronidazole(G4) showing still of focal area of necrosis(Ne) with inflammatory cell(In) infiltration , increase kupffer cells(K) and sinusoid (Si) dilation , (10x.H&E).

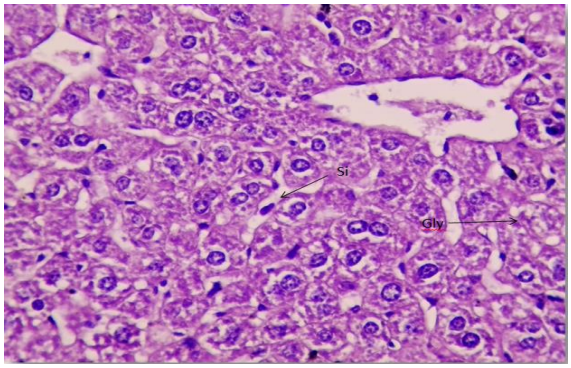
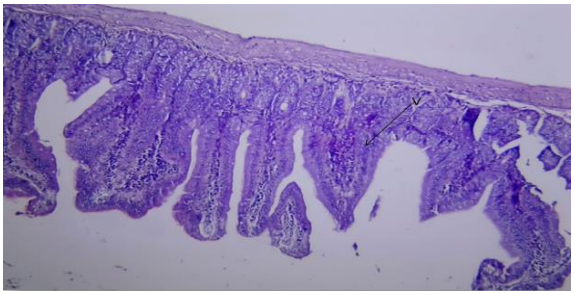
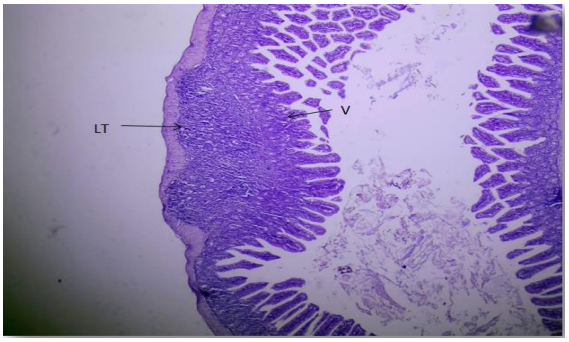


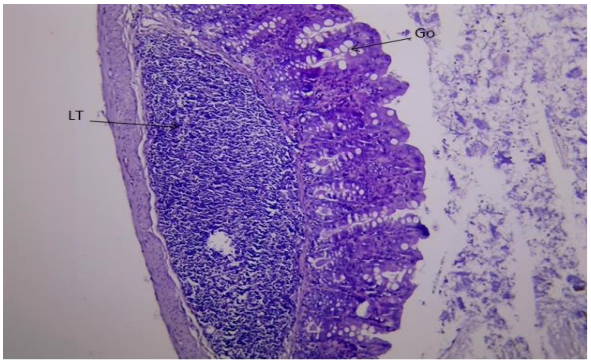
Figure (5) : A Section of liver of mice that treatment with metronidazole and contraceptive pills (G5)showing mild depilation of Glycoprotine (Gly) and sinusoid(Si) dilation, (10x.H&E).



Figure(6) : A section of intestine of mice control negative (G1) was showing normal histological appearance of intestinal villi (V) with normal appearance of submucosa gland (10x.H&E).



Figure(7) :A section of intestine in mice that infected with E.histolytica (control positive G2) showing of hyperplasia of lymphoid tissue (LT) (peyers patch) with shortening villi (V), (4x.H&E).



Figure(8) : A section of intestine in mice that infected with E.histolytica and treatment with contraceptive pills(G3) showing of hyperplasia of Goblet cell (Go) with Lmphoid tissue hyperplasia (LT),(4x.H&E).

Figure (9) :A section of intestine in mice that infected with E.histolytica and treatment with metronidazole (G4) showing of retain the shape of intestinal villi(V) looks like to near the normal but still with Goblet cell hyperplasia(Go), (10x.H&E).

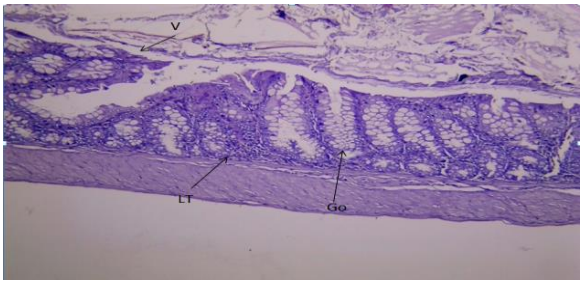


Figure (10) : A section of intestine in mice that infected with E.histolytica in(G5) showing that wide of intestinal villi(V) with hyperplasia of Goblet cell and normal appearance of lymphoid tissue (LT)(payer's patch), (10x.H&E).

Discussion

The protozoan parasite Entamoeba histolytica is the causative agent of amebiasis, an infection that manifests as colitis and, in some cases, liver abscess (Uddin et al.,2021).The motile form of E. histolytica, the trophozoite, lives in the lumen of the large intestine, where it multiplies and differentiates into the cyst, the resistant form responsible for the transmission of the infection(Espinosa-Cantellano& Martinez-Palomo.,2000). The majority of trophozoites were found attached to inter glandular epithelium, and the trophozoites were associated with micro ulcerations of the tunica mucosa associated with thinning of the mucus layer, shortening of the microvilli, bleeding, degradation of the extracellular matrix, cell vacuolation, necrosis, hemorrhage, and compression and distortion of individual cells due to the presence of large numbers of trophozoites (Ralston et al .,2011). In the initial stages, trophozoites produce nonspecific lesions characterized by thickening of the mucosa, due to glandular hyperplasia and stromal edema, the edematous thickening of the mucosa, the glandular hyperplasia, and the stromal edema described in the nonspecific lesion are probably due to mild irritation of the epithelium produced by soluble amebic products. Trophozoites in axenic cultures are known to secrete a variety of molecules, some of which have been identified as proteases. Irritation stimulates goblet cells to release mucus, at the same time that it increases its production, thus explaining the glandular hyperplasia(Espinosa-Cantellano& Martinez-Palomo,. 2000).In the United Kingdom, approximately 26% of women of reproductive age use hormonal contraception(Firman et al.,2018) with combined oral contraceptive pills (COCPs) being the most popular method. COCPs work by releasing an estrogen and a progesterone. Numerous studies have found an increased risk of developing IBD in association with oral contraceptive pill use (Cornish et al .,2008;Ortizo et al ,.2017;Wang et al .,2019) . When E. histolytica trophozoites enter the liver, they form their own abscesses, which are well-defined regions of cytolysis liver cells, liquefied cells, and cellular debris. The lesions are encased in connective tissue, which contains a few inflammatory cells and trophozoites. Nearby parenchymal cells are frequently unaffected. However, E. histolytica trophozoite lysis of neutrophils may release mediators that cause liver cell death and extend damage to hepatocytes not in direct contact with the parasite (Stanley, 2003).The ability of amoebae to destroy host tissue and survive in the liver is accompanied by a strong adaptive response and regulation of proteins, such as amebic virulence factors (Bruchhaus et al., 2002). This is consistent with what was said Carranza-Rosales et al. (2012) amoeba that induce the programmed death of hepatic cells and noted that it increased in number the progress of infected time.In G3 we showed lipid in lever that caused because utilized the contraceptive pills ,these correspond with studies in Ghana, most women reporting to family planning clinics use Depo-Provera (depot medroxyprogesterone acetate [DMPA]), an injectable contraceptive (IC), with a few depending on OCs. DMPA is said to increase LDLC and decrease HDLC.(Enk et al., 1992),these increased of invasion of E .histolytica as the studies Pathogenesis of E. histolytica is characterized by sequential steps as mucus layer degradation, adherence to the epithelium, cytotoxic, and cytolytic events, phagocytosis, migration, and tissue invasion (Espinosa-Cantellano and Martinez-Palomo, 2000).All these processes are performed with the active participation of lipids (Espinosa-Cantellano and Martinez-Palomo, 2000; Byekova et al., 2010; Das and Nozaki, 2018), thanks its lipid composition, amebic membranes are resistant to the lytic compounds of the parasite (Andrд et al., 2004).In other studies founded; an increased risk of IBD with increased duration of exposure to COCPs. Not only were progestogen-containing pills associated

with Crohn's disease but there was a modest association with ulcerative colitis. There was no association between parenteral progestogen-only contraceptives and IBD. These results are generally consistent with the hypothesis that the estrogenic component of contraceptives may lead to the pathogenesis of IBD (Pasvol et al.,2022). other study for COC looked at the link between hormonal contraceptive use and the development of autoimmune diseases, in which the immune system turns against the body and causes organ damage(Williams ,2017). Also reported that oral contraceptives and estrogens are both associated with several liver-related complications, including sinusoidal dilatation, intrahepatic cholestasis, hepatic adenomas, peliosis hepatis, hepatocellular carcinoma, hepatic venous thrombosis, and an increased risk of gallstones, as demonstrated by(Olry et al. ,2020). Ponnatapura et al.,(2019) also demonstrated that oral contraceptive pill hepatic complications include intrahepatic canalicular cholestasis, vascular pathologies, and neoplasm formation. While it is unclear whether estrogen plays a role in focal nodular hyperplasia, hamartoma, or hemangioma, oral contraceptive pill use is strongly linked to hepatic adenomas.Oral administration of metronidazole has shown side effects such as vaginal discharge, symptomatic candidiasis, vulvovaginal irritation, gastrointestinal disturbances, nausea, and metallic taste. The potential for the emergence of metronidazole-resistant strains of E. histolytica has led to the development of novel treatment strategies against amebiasis (Bansal et al., 2006). It has been shown that hepatic invasion by E. histolytica increases oxidative stress and phase-cytokine production through activation of nuclear factor kappa B (NF−B) in favor of progression of liver damage (Aldaba-Muruato et al., 2017).Nitroimidazoles are the only treatment for invasive amebiasis. Nitroimidazoles are toxic drugs, and resistance to them has developed in other anaerobic protists (Shirly et al.,2018) This explains our vision of the spread of inflammatory cells in the liver and goblet cells in the intestines.

Conclusion

- According to the study conducted the COC increased the female sex hormone in female mice
- COC it increased from virulence of the intestine and liver infection with parasite compare with other groups

Refreance

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