



Evaluation of Allicin Supplement in Amebiasis - Compromised Pregnancy for Rats with Experimental Infection

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Abstract

Amebiasis is the third most common parasite-related cause of death globally caused by the protozoan *Entamoeba histolytica*. The presence of parasites during pregnancy has been linked to hazards for pregnancy and undesirable outcomes. Little is known about their impact on the progression of pregnancies. This study aims to reveal the influence of amebiasis incidence content on pregnancy before and after treatment with allicin. Female rats were divided into five groups of eight rats each, group (1) was given distilled water as a control, while groups 2, 3, 4, and 5 were infected with suspension *E. histolytica*. Every rat delivers cysts per milliliter via oral administration to patients at the Bint AL-Huda teaching hospital in Thi-Qar province of southern Iraq. and the group (3) was treated orally with metronidazole (20) mg/kg. group (4) gives allicin after infection at a rate of 0.3 mg/kg. group (5) gives allicin and metronidazole after infection. rats were placed for mating, and after confirming pregnancy and determining the first day of pregnancy, they were killed by ether and explained in the last trimester of pregnancy for gross and histological examinations. The results showed that group 2 had a significant reduction in uterine and ovarian weights ($P \leq 0.01$) when compared to groups g1, g3, g4, and g5. Histological findings revealed no pathological lesions in the genitalia of all the rats. There were significant differences between groups in When hormone levels were measured ($P \leq 0.01$) there was an improvement in female reproductive abilities by increasing the birth rate in groups 1, 3, and 4 compared to group 2. These results indicate that allicin improved the reproductive capabilities of female rats.

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Introduction

Protozoan parasites are microscopic, unicellular microorganisms that have a high prevalence and incidence worldwide, making them a major health risk. Their pathological changes are, as wanted to follow, strongly associated with pathogenic effects and their symptoms' host's location in tissues, intestinal or blood (Paul *et al.*, 2020). *Entamoeba histolytica* is a nonflagellated protozoan parasite that produces pseudopods and causes amoebic dysentery in humans. The infection may be asymptomatic or the infection may develop into more serious pathological signs such as amoebic colitis and amoebic liver abscess. Amoebic dysentery

is an indicator of prevalence and mortality worldwide, with measured estimates that it kills about 55,000 people (Shirley *et al.*, 2018). During his life cycle, the parasite has two main stages: the Trophozoite feeding stage and the Cyst stage. The last stage represents the infectious stage. Infection begins with ingestion of mature cysts present in contaminated water and food for the infectious stage (Cuellar Guevara, 2019). The parasite can infect different organs, such as the intestines, liver, lungs, pancreas, and brain, causing extraintestinal amoebic disease (Siddiqua, 2016).

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Amebiasis can affect pregnant women and be more severe, as it causes symptoms such as bloody diarrhea, vomiting, and fever, and thus leads to premature birth of the fetus due to the immune deficiency of the pregnant mother (Karadbhajne *et al.*, 2020). Pelvic pain, salpingitis, tubo-ovarian abscess, and genital ulcers are all symptoms of *Entamoeba histolytica* infection and plays an indirect influence on infertility. *E. histolytica* has been associated with salpingitis (Calore *et al.*, 2002) ulcerative vulvovaginitis (Citronberg and Semel 1995), endometrial infections (Azmi and Othman, 2021) and in 1993, a uterine carcinoma was replicated (Mhlanga *et al.*, 1992) genital abscess, tubo-ovarian abscess, and ulceration in women that may be associated with infertility (Niederhauser *et al.*, 2007). Pregnancy is a physiological condition that is also usually associated with changes in disease risk. Resistance to fetal infections is produced by a change in immunity during pregnancy. It's also related to a decrease in immunity during pregnancy, pregnant women in terms of their children's health, both the mothers and their children (Dagnaw *et al.*, 2021) One of the active components in freshly crushed garlic (*Allium sativum*) homogenates, Allicin [S-(2-propenyl)2-propene-1-sulfinothioate], has been found to have antimicrobial and antitumor capabilities. Allicin's growth-inhibitory activity on *Entamoeba histolytica*, the protozoan parasite that causes more than 50 million symptomatic incidences of intestinal amebiasis each year, was particularly noteworthy. The antimicrobial properties of allicin have been attributed to its specific interference with sulfhydryl enzymes (Gruhlke *et al.*, 2019). This study was undertaken to explore potential of the allicin supplement in amebiasis during pregnancy on some aspects of female fertility using rats as a model.

Material and Methods

Samples Collection

Samples were collected from the stool of certain parasite-infected patients at Bint Al-Huda teaching hospital in Thi-Qar province, south of Iraq, to acquire the parasite *Entamoeba histolytica* and its Trophozoite and Cyst stages parasites. The samples were placed in sterile plastic bottles and brought directly to the laboratory for examination. Preparation suspension solution from the parasite *Entamoeba histolytica*. (Clark and Diamond, 2002) for injection to animals.

Infection of Animals

The study was conducted on female white rats of the type (*Rattus norvegicum*) were divided into five groups, with an average weight of approximately 260–230 gm, with an average age of (14-16) weeks. The animals were bred in special cages in the Animal House Department-College of Education for Pure Science/The Qar University. The animals were divided as follows:

- The first group: control group was treated with normal saline NaCl (0.5 ml).
- The second group was dosed with neutral physiological saline after infection with *Entamoeba histolytica* as a positive control group for 20 days.
- The third group: metronidazole (20 mg) dissolved in distilled water after infection at a rate of (20) mg/kg per animal for 20 days.
- The fourth group was dosed with allicin after infection at a rate of 0.3 mg/kg for each animal for 20 days.
- The five groups were dosed with allicin (0.3) mg/kg and metronidazole (20 mg) for 20 days.

Appearance of infection in animal feces was determined after 15 days. The animals were placed for mating, and after confirming pregnancy and determining the first day of pregnancy, they were killed with ether and annotated in the last three months of pregnancy to study the histological changes in the ovaries and to measure reproductive capabilities and hormones in rats.

Blood samples: were taken from pregnant mothers on the 19th day of pregnancy in order to measure hormones (PROG, HCG, AFP) using CL Electrochemiluminescence technology (Cobas 411 E), (Nawaz, 2011). All data were represented as mean \pm SE. results analyses by using (GraphPad Prism 8) software one way ANOVA, (Tykey test).

Results and Discussion

The mean \pm SE of the weights of the female reproductive organs (uterus and ovaries) is shown during this study, as statistically significant differences in weights between groups were recorded.. Significant differences at $P \leq 0.01$, Figure (1, 2).



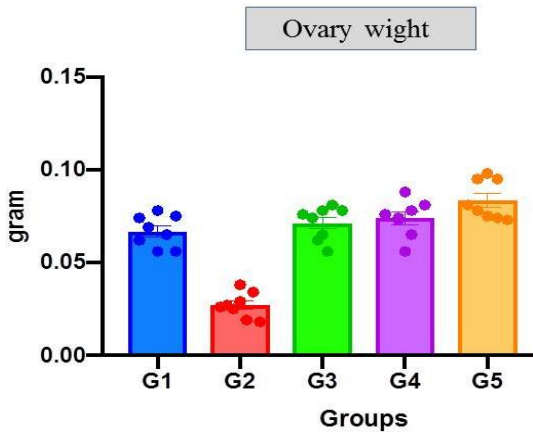


Figure 1. Ovarian wight in all study groups. Significant differences at $P \leq 0.01$

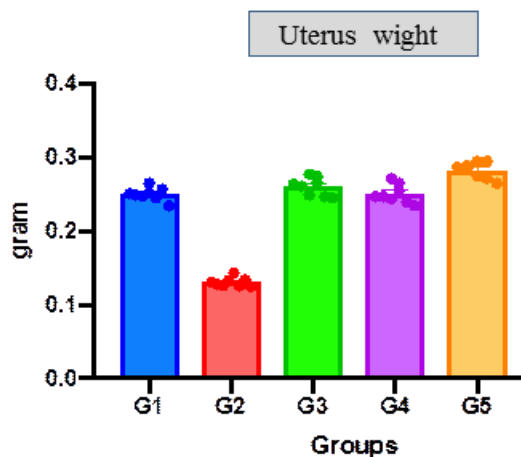


Figure 2. Uterus Wight in all study groups. Significant differences at $P \leq 0.01$

Histological Changes in the Ovary

Fig: 3 G1 The appearance of primary and secondary ovarian follicles, as well as the corpus luteum, as opposed to Fig: 4 G2 infection with *Entamoeba histolytica*, which was characterized by low primary and secondary ovarian follicles and oocyte degeneration Fig: 5 G3 showed primary and secondary ovarian follicles. Fig: 6 G4 was characterized by an increased appearance of primary and secondary ovarian follicles and corpus luteus. Fig: 7 G5 was demonstrated different mfollicles at various stages of development; primary, secondary, and matured (Graffian) follicles. There were no pathological lesions.

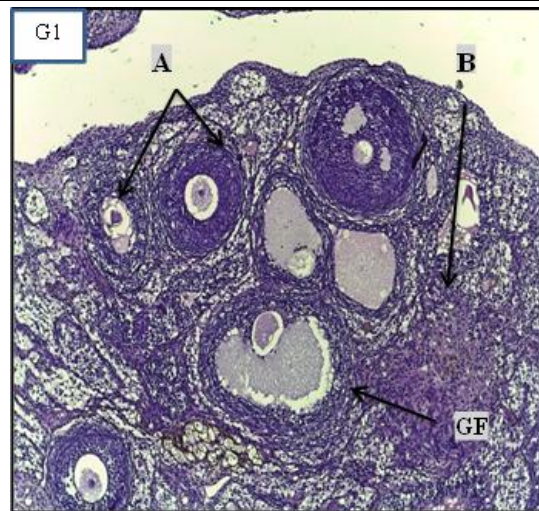


Fig. 3. Photomicrograph of ovary of rat (A) primary and secondary ovarian follicles (B) corpus luteus (GF) Graffian follicle 10x (H&E)

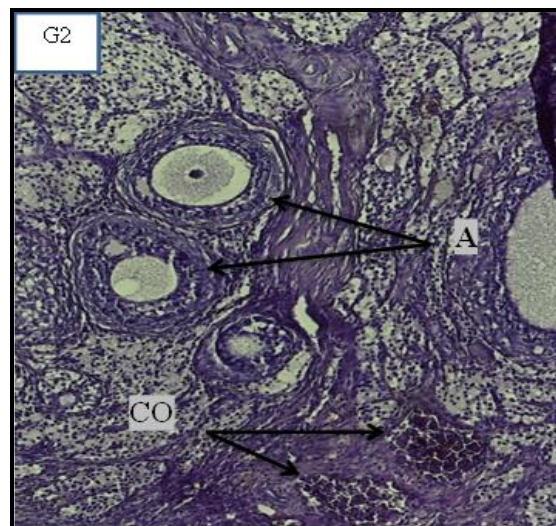


Fig. 4. Photomicrograph of ovary of rat (A) primary and secondary ovarian follicles (CO) congestion 10x (H&E)

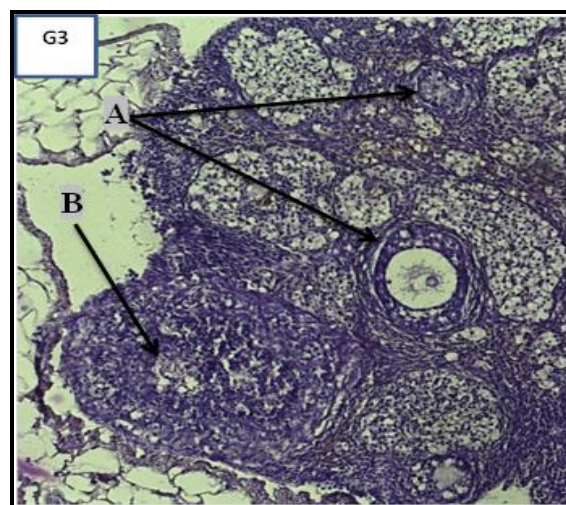


Fig. 5. Photomicrograph of ovary of rat (A) primary and secondary ovarian follicles (B) corpus luteus 10x (H&E)



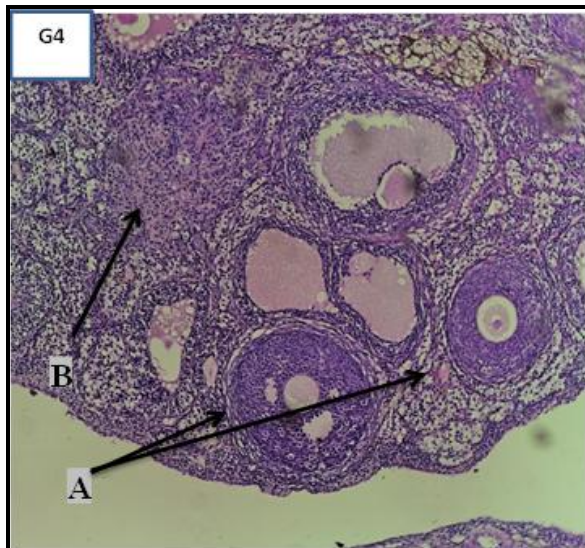


Fig. 6. Photomicrograph of ovary of rat (A) primary and secondary ovarian follicles (B) corpus luteus 10x (H&E)

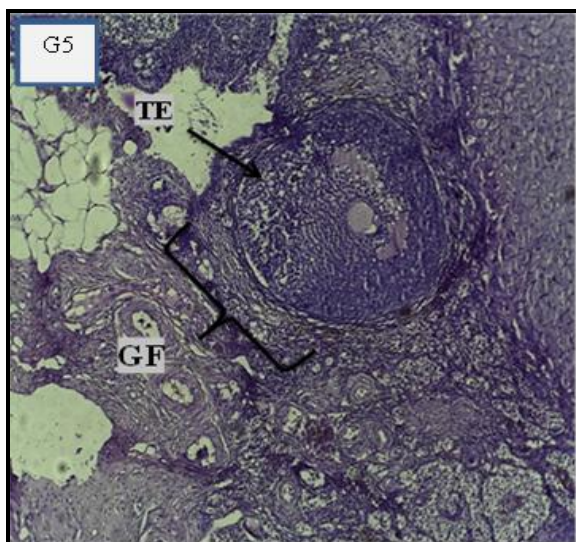


Fig. 7. Photomicrograph of ovary of rat (GF) Graffian follicle (TE) Theca externa 10x (H&E)

When comparing the level of hormones in the study groups, significant differences ($P \leq 0.01$) were observed in the levels of hormones (PROG, HCG) as the level of hormones decreased in the affected group than the rest of the groups, and an improvement was noted in the affected group treated with allicin and metronidazole. The alpha-fetoprotein did not show significant changes in the study (Figure 6,7,8).

The Reproductive Capabilities of Females

The table (1) shows the reproductive capabilities of females and the rate of pregnancy during For females during the period of infection and

treatment, as the table showed significant differences in the number of births ($P \leq 0.01$).

Table 1. Reproductive capabilities of females and the rate of pregnancy

Groups	No. of females	No. of pregnant females	No. r of births (fetal rate) Mean \pm SD	Fertility rate %
G1	8	8	7.62 \pm 0.37	100
G2	8	3	1.37 \pm 0.67	37.5
G3	8	5	4.87 \pm 1.44	62.5
G4	8	6	4.7 \pm 1.08	75
G5	8	7	7.62 \pm 1.11	87.5

Discussion

Intestinal parasitic infections are spreading worldwide with increased travel and movement around the world from contaminated foods. These infections are not entirely limited to traditionally endemic areas. The prevalence of parasitic infection in endemic areas among pregnant women ranges from 24 to 70% with approximately 10% of the women infected with multiple parasites during pregnancy, it comes with an increase in nutritional requirements during this period coupled with the immune changes of the body, all factors that lead to the acquisition of parasitic infection, which may be associated with negative results such as anemia, which may contribute in some cases to an increase in the death rate, and the presence of infection may lead to Parasitic worms during pregnancy also lead to immune diseases that can contribute to Increasing the disturbance of the health of the pregnant woman and thus affecting the maternal immunity and the immune system response in the child after birth. However, safety concerns have not been raised with the use of drugs recommended during pregnancy to reduce parasitic infection, which may disrupt the pregnant woman's health and thus affect maternal immunity and the immune system response in the child after birth (Mohan *et al.*, 2020). The current study revealed that infection with the parasite *Entamoeba histolytica* affects the weight of reproductive organs by affecting the total weight of the animal and that giving allicin compound with the drug metronidazole will improve the effect of infection. This is consistent with Thakur *et al.*, (2009) as it was found that the extracts of *Carum carvi* and *Curcuma longa* significantly increased the uterine body weights of treated mice. The result varies according to the condition found Raji *et al.*, (2012) decrease in



uterine body weights as a result of the garlic extracts. Rats given the drug and allicin had a higher rate of successful mating and conception. This agrees with the researcher's findings in giving doses of an aqueous extract of *Stevia rebaudiana* bertani. Ezumi *et al.*, (2007). It was shown Falahatian *et al.*, (2022). In our current study, the histological images showed the presence of some histological changes in the structure of the ovaries infected with *Entamoeba histolytica*, such as congestion in blood vessels, and normal growth was observed in the tissue sections treated with sine. This agrees with Raji *et al.*, (2012) if the absence of pathological lesions was recorded in the treated ovaries with different concentrations of garlic extracts. The results of the current study showed a significant decrease in Progesterone and HCG hormone in the affected group compared to the control group. The explanation for this is that due to the high incidence of infection and the low incidence of pregnancy, and the small number of placentas that have a role in the formation of the HCG hormone, which leads to a decrease in its concentration in the blood serum. (Gangstad *et al.*, 2012). While there were no significant differences in the alpha fetal protein, which indicates that there were no abnormalities or disorders in fetuses during pregnancy. That treatment with one of the garlic compounds is also related to fertility after treatment with R10 fraction in PCOS. as he clarified The R10 portion of garlic has immunomodulatory effects that relieve PCOS symptoms This is consistent with our current study in that allicin contributes to raising the fertility of rats. Premature birth increases the risk of developing anemia and amebiasis during pregnancy. This is what was found (Mahande and Mahande, 2016) and this is consistent with our current study with the decrease in the number of fetuses and the loss of most of them during parasitic infection.

Conclusion

Infections with intestinal parasites during pregnancy and their consequences on fertility and negative effects are good evidence of that. The role of allicin is that it has no detrimental effect on the reproductive performance of rats. Allicin is a relatively new compound for treating intestinal parasites and increasing female fertility and requires further investigation to validate it.

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