Estimation of some antioxidants and liver enzymes parameters in rats infected with Entamoeba histolytica and the extent of the therapeutic effect of gold nanoparticles

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Entamoeba histolytica, gold nanoparticles, Glutathione (GSH), Malondialdehyde (MDA), Aspartate amino transfrase (AST), Alanine amino transfrase (ALT).

ABSTRACT
This study was conducted on patients visiting Tikrit Teaching Hospital in Tikrit city and visiting some private laboratories in Al-Alam district for the period from the beginning of January 2023 to the end of December 2023. 548 stools were collected and the percentage of infection with the amoeba Entamoeba histolytica was 29.92% for 164 samples positive for microscopic examination. The study included the biochemical aspects that studied the effect of infection with the amoeba parasite on some antioxidants of infected rats treated with different concentrations of gold nanoparticles. It was noted that a significant increase occurred in infected rats treated with different concentrations of gold nanoparticles when compared with the control group at a probability level of 0.01 in Glutathione (GSH), Malondialdehyde (MDA). The aspects that studied the effect of infection with the amoeba parasite on some liver enzymes of infected rats treated with different concentrations of gold nanoparticles, it was noted that there was a significant increase in infected rats treated with different concentrations of gold nanoparticles when compared with the control group at a probability level of 0.05 in (AST) Aspartate amino transfrase, Alanine ameno transfrase (ALT).

1. Introduction

Entamoeba histolytica is an intestinal parasitic protozoan that causes amoebic dysentery in humans, which is a major cause of death and is responsible for approximately 100,000 deaths annually, as the number of people infected with the parasite is estimated at about 480 million infections worldwide. Despite its generally poor quantitative estimate, the impact of amoebiasis is still significant, and it is considered a prevalent disease, as estimates reach 40% in some population groups in some countries such as India and China (Nasar, 2024; Guillén, 2023). Amoebic dysentery is most prevalent in tropical and subtropical regions of the world and in developed countries, where it is common among travelers and new immigrants (Norman et al., 2020).

The life cycle of the E.histolytica parasite is simple, as its life cycle goes through two main stages: the traphozoite and the cyst. Infection with the parasite occurs by eating food and drink contaminated with the cyst in unsanitary conditions (Sharif & Raouf, 2022; Guillen, 2021). The stage that causes infection and causes damage to the host is the feeding stage, which lives in the cavity of the infected person's large intestine and feeds on the mucous membrane lining the intestine, damaging its cells, causing painful cup-shaped ulcers. This causes amoebic dysentery (ChIDEbElu & NwEzE, 2020). As for the clinical symptoms of infection with the disease, some people infected with the parasite do not show symptoms of the disease, while others show symptoms of the disease, represented by abdominal pain, diarrhea, colitis, and the appearance of mucus and blood in the stool (Siciliano et al., 2020).

Gold nanoparticles (AuNPs) have optical and electrical properties that are different from those of conventional materials and have a bright future in medicine. These properties include stability, surface plasmon resonance, surface chemistry, multifunctionality, and high surface area-to-volume ratio. The size of AuNPs makes it easy for them to pass through cell membranes and affect cell permeability, protein synthesis, and metabolism, causing cell death in microbes (Alnuaimi, et al., 2023).

Glutathione (GSH) is a unique molecule essential for life that is involved in key aspects of cellular homeostasis, and has a fundamental role in defending against oxidative damage that occurs during all different diseases (Labarrere & Kassab, 2022).

Malondialdehyde (MDA) is widely used as a biomarker for assessing oxidative stress in biomedical fields. Lipid oxidation is a sequential phenomenon that leads to the formation of various active compounds that lead to cell damage. Biomonitoring of malondialdehyde has been used in in vivo and in vitro studies as a key biomarker for various disease patterns including hypertension, diabetes, atherosclerosis, heart failure and cancer (Singh et al ., 2014).

Aspartate amino transfrase(AST) plays a key role in amino acid metabolism, maintenance of the NAD+/NADH ratio in cells, Krebs cycle activity, purine/pyrimidine base synthesis, urea and protein synthesis, and gluconeogenesis. Serum AST activity is increased in patients with acute myocardial infarction in proportion to the extent of myocardial necrosis. (Ndrepepa, 2021).

Alanine ameno transfrase (ALT) is involved in amino acid metabolism, catalyzing the transfer of the alpha-amino group from alanine to alpha-ketoglutaric acid. Serum ALT levels are elevated due to hepatocellular damage, i.e. “cellular degeneration,” and are not necessarily associated with inflammation or hepatic steatosis. Conversely, by definition, 5% of healthy individuals have abnormal serum alanine aminotransferase levels (Lonardo, 2024).

2. Methodology

Samples collection: Stool samples for E. histolytica were taken from patients who visited and were hospitalized at Tikrit Teaching Hospital and private laboratories in Al-Alam District, who were suffering from severe to moderate diarrhea, and most often those who had bloody diarrhea, during the period from 1/1/2023 to 30/12/2023. The samples were placed in sterile plastic bottles with a wide opening and a tight lid to maintain the moisture of the sample and prevent it from drying out. Microscopic examination of the samples was performed during the first half hour, taking care to take the sample from areas containing mucus or blood in search of the active stage responsible for infection with the parasite. The active stage is often observed in soft samples and is rarely observed in solid or semi-solid stool, which usually contains the cystic stage, and its presence often indicates infection with the parasite (Clark & Diamond, 2002).

2-2: Examination of stool samples: The samples were examined by the direct wet smear method used to diagnose the trophozoite stage to observe the movement of the amoebic dysentery parasite, which can be distinguished from the movement of other types of amoebae in that its movement is always directional. It is possible to observe the parasite cytoplasm containing red blood cells (R.B.C.). Especially in the samples of acute amoebic dysentery that were collected, the examination is done by taking a drop of physiological saline solution and placing it on one end of the glass slide and a drop of local iodine solution on the other end of the slide, and an amount of feces was taken the size of a match head from several places by means of a clean wooden stick, and the amount of feces taken was mixed with the physiological saline solution on the slide, and in the same way another amount of feces was taken and mixed with the local iodine solution until the mixture became homogeneous, and then the slide cover was placed and then examined with a light microscope with a 100X oil lens to confirm the presence of the parasite (Singh et al, 2009).

2-3: Parasite isolation: The parasite was isolated from stool samples obtained from patients with diarrhea, in which the presence of the parasite was proven by direct microscopic examination. The parasite cysts were concentrated by flotation with a saturated sugar solution prepared according to the method of (John & Petri, 2006), consisting of dissolving 500 g of sucrose powder in 1000 ml of distilled water, then 6.5 g of phenol was added to the solution. The method is done by mixing a certain weight of stool in 10 cm of distilled water, then the mixture was filtered through several layers of sterile medical gauze until impurities were removed. The filtrate was placed in glass tubes and placed in a centrifuge to be centrifuged at a speed of 2500 rpm for 10 minutes. The filtrate was then discarded and the precipitate was taken and 3 cm3 of distilled water was added to it and centrifuged at a speed of 2500 rpm for 10 minutes. The process was repeated until a completely clear suspension was obtained. Then The filtrate was discarded and the sediment was taken and an appropriate amount of saturated sugar solution was added to it until the tube was full and placed in the centrifuge at a

speed of 500 rpm for 10 minutes. Then the upper layer was examined to calculate the number of bags given to the animal (Clark and Diamond, 2002)

2-4: gold nanoparticles: Gold nanoparticles (GNPs) were prepared by chemical synthesis, where citrate-coated gold nanoparticles (GNPs) with a diameter of 20 nm were prepared by reducing 1 mmol of HAuCl4 with sodium citrate (1%) according to the method of (Herizchi et al., 2016; Leng et al., 2015) with slight modification as follows:

1- 10 -1ml of HAuCl4 was heated near boiling temperature.

2- Add 1 ml of citrate solution quickly.

3- The solution was stirred continuously with a magnetic stirrer, and the yellow color of gold ions faded to colorless. Then the sky-blue color appeared and darkened to dark blue, and turned to cherry red after 15 minutes of heating.

4- The mixing continued until cooling to room temperature.

5- The dark red mixture was filtered with a 0.22 μmol membrane filter (Millipore), then the gold nanoparticles were stored in the refrigerator at 4°C until use.

2-5:Experimental animals: Male laboratory rats, 6-8 weeks old and weighing 120-180 grams, were used. They were taken from the animal house of the College of Veterinary Medicine / Tikrit University, after ensuring that they were free from apparent diseases and free from intestinal parasites. The animals were placed in clean plastic cages for raising laboratory animals. The animals were divided into four groups and placed in plastic cages, one cage for each group. All of them were in large cages equipped with special feeders and covered with a metal cover with a floor covered with sawdust. The animals were treated as follows:

1- The first group (negative control): includes (5 animals) and was given normal drinking water throughout the experiment period.

2- The second group: positive control includes (5 animals). The rats were orally dosed with 8000 cysts of histolytic amoeba and the stool of each rat was examined for a week until the infection appeared.

3- The third group: includes (5 animals) orally dosed with histolytic amoeba and the stool of the rats was examined daily for a week until the infection appeared, then they were orally dosed with gold nanoparticles at a concentration of 3.75 cm3 after infection for 10 days at a rate of 1 ml.

4- The fourth group: includes (5 animals) orally dosed with histolytic amoeba and the stool of the rats was examined daily for a week until the infection appeared, then they were orally dosed with gold nanoparticles at a concentration of 5 cm3 after infection for 10 days at a rate of 1 ml.

Laboratory rats were dosed with 8000 amoeba cysts orally using a gastric syringe, by inserting the syringe into the mouth, and pushing the liquid containing the parasite cysts. For a week after infection, the rats were placed in clean cages free of sawdust. The parasite cysts were investigated in the feces of infected animals to confirm the occurrence of parasite infection. The infection was confirmed by preparing several smears from the feces of infected rats on a glass slide and examining them under a microscope. The parasite and its different stages were observed. After confirming the infection of the animals, they were dosed with gold nanoparticles. The rats were dissected 10 days after treatment with gold nanoparticles, then examined to observe the pathological changes visually. The intestines and liver were taken and fixed in formalin solution, and preserved in it to study the histological changes during the infection period.

2-6: Collect Blood Samples: After the specified period of the experiment, the rats were killed after anesthetizing them with chloroform. Then, blood samples were drawn from the heart and placed in test tubes free of anticoagulant. They were left for approximately 15 minutes at 37°C. After that, the serum was obtained by centrifuging at 3000 rpm for 15 minutes. It was stored at -20°C in new, clean

plastic tubes until the biochemical tests were performed, which included antioxidants (MDA, GSH) and liver enzymes (AST, ALT).

2-7: Antioxidant Tests

1- Determination of serum GSH concentration: The serum GSH concentration was estimated using SUNLONG Company in China.

2- Determination of serum MDA concentration: The serum MDA concentration was estimated using SUNLONG Company in China.

2-8: Biochemical tests

1- Determination of the concentration of the enzyme (AST) in the blood serum: The concentration of the enzyme (AST) in the blood serum of laboratory rats was measured using several special analyses from the English company Randox.

2- Determination of the concentration of the enzyme (ALT) in the blood serum: The concentration of the enzyme (ALT) in the blood serum of laboratory rats was measured using several special analyses from the English company Randox.

2-9:Statistical Analysis: The results were statistically analyzed using the (ANOVA) program, using the Chi-Square (Chi X2) test, at a probability level of ($P \geq 0.01$) and ($P \geq 0.05$) to determine the significant differences in infection with the parasite under study and its relationship to some biochemical parameters when compared with the control groups

3. Results and discussion

Percentage of E. histolytica parasite in stool samples examined under a microscope: Stool samples were collected from the laboratories of Tikrit Teaching Hospital and some private laboratories in Al-Alam District for both sexes for the period from January 2023 to December 2023. The E. histolytica parasite was diagnosed through microscopic examination of stool samples in only 164 samples out of 548 samples, at a percentage of 29.92% (Table 3-1).

Table (3-1): Percentage of samples positive for *E. histolytica.*

Total number of samples tested	**Number of infected samples**	**percentage**
548	164	**%29.92**

This study recorded rates close to those recorded by (Anwar, 2014) in Kirkuk city, where the infection rate reached 30.22%, and what (Nassar et al., 2019) recorded in Basra Governorate, where the infection rate reached 32%. It also agrees with what (Al-Sumaidi, 2022) recorded in Tikrit, where the infection rate was 26.1%. And with what (Mohsin et al., 2022) recorded in Kirkuk, where the total infection rate with the E. histolytica parasite reached 29.6%. While our results did not agree with what (Lazar, 2012) recorded, where the infection rate reached 21.67% in Kirkuk, and (Al-Yasiri, 2015) recorded in Nasiriyah a rate of 19.5%, and (Kadir et al., 2018) in Tikrit recorded an infection rate of 9.3%. What was recorded by (AL-Khikani et al., 2019) in Babylon, where the infection rate reached 17.91%, and ((Zangana & Erdeni ., 2020) recorded in Tikrit an infection rate of 13.375%, and with what was recorded by (Aziz et al., 2022) in Sulaymaniyah, he recorded an infection rate of 19.3%, and (Nayyef et al., 2022) recorded in Baghdad an infection rate of 15.89%.

The differences in the incidence of the parasite in this study and previous studies are due to differences in population density, sanitation, personal hygiene, geographical location, climatic conditions, number of samples examined, accuracy and skill of the examiner, educational level and age groups in the population studied. As for the similarity in the incidence rate, it may be due to similarity in cultural, health and social levels (Zangana & Erdeni, 2020).

3-2: Antioxidants (GSH and MDA concentrations): The results showed a difference in the

concentration rate of antioxidants GSH and MDA in laboratory rats infected with dysentery amoeba and treated with concentrations of 3.75 and 5 cm3 of gold nanoparticles, as a decrease in the concentration rate of GSH was observed for the positive control group, which reached 13.447 ng/cm3, compared to the negative control group, which reached 13.870 ng/cm3, and an increase in the concentration rate of GSH was observed when using gold nanoparticles at a concentration of 3.75 and 5 cm3 for the infected groups.

It was also noted that there was an increase in the MDA concentration rate for the positive control group, which reached 38391 ng/cm3, compared to the negative control group, which reached 25083 ng/cm3. It was also noted that there was a decrease in the MDA concentration rate when using gold nanoparticles at a concentration of 3.75 and 5 cm3 for the infected groups (Table 3-2).

Table (3-2): Average concentrations of antioxidants GSH and MDA in laboratory rats infected with dysentery amoeba and treated with different concentrations of gold nanoparticles

Groups / **Tests**	**Mean ± S.D**	
	MDA	**GSH**
negative control	25083±618	**13.870±0.348**
positive control	38391±724	**13.447±0.402**
Gold nanoparticles 3.75 cm3	31482±820.1	**14.037±0.0523**
Gold nanoparticles 5 cm3	33530±389	**15.349±0.0778**
Statistical analysis	F-Value = 2.84** P-Value = 0.017	**F-Value = 3.17** **P-Value = 0.010**

The mark ** indicates the presence of significant differences at the level of $P \leq 0.01$.

These results are consistent with the study of (Mohsin et al., 2022) in Kirkuk, which showed a significant decrease in GSH concentration activity (P<0.01) compared to the group infected with the amoeba parasite and the healthy group. Our study also agrees with the study of (Hamad, 2021), as the infected group showed a decrease in GSH concentration to about (5.125±1.137) mmol/g compared to the normal control group (7.189±1.885) mmol/g, which is considered highly statistically significant (p<0.001).

Glutathione is an important component of the body's antioxidant system and intracellular protective mechanisms against many internal and external cues that lead to a state of oxidative stress. It is an endogenous antioxidant that acts against oxidants such as some drugs, carcinogens, and other pathological injuries. Low concentrations of glutathione make tissues more receptive to oxidation (Mohsin et al., 2022).

The reason for the increase in GSH in those treated with gold nanoparticles is due to oxidative stimulation. Gold nanoparticles may stimulate antioxidants or stimulate the oxidation process within cells, leading to increased production of glutathione as a defense mechanism against toxic or oxidative effects. Or as a result of the effect of gold nanoparticles on cell balance and increasing the production of glutathione to help protect cells from the potential toxic effects of nanoparticles.

As for MDA, it is one of the products of lipid peroxidation, an indicator of oxidative damage caused by free radicals, and plays a role in the pathogenesis of many parasitic infections (Hamad, 2021). In this study, compared with the normal control group, the MDA concentration of the infected control group increased significantly. The increase in MDA concentration observed in this study may be due to the excessive production of free radicals and oxidants after infection, or it may indicate a decrease in the enzymatic activity of the antioxidant defense system. This result is consistent with the aforementioned results, as our study agrees with (Al-Badri, 2023) in Samarra on the Giardia parasite, which recorded an increase in the positive control group reaching 233.313 ng/cm3, compared to the control group, which reached 21.412 ng/cm3, and these results are consistent with the study (Mohsin et al., 2022) in Kirkuk, which showed a significant increase in the concentration of MDA (P<0.01) compared to the group infected with the amoeba parasite and the healthy group, and also agrees with

the study (Hamad, 2021), as the infected group showed an increase in the concentration of GSH to about 14.271 mmol/g compared to the normal control group 9.435 mmol/g, which is considered highly statistically significant (P<0.001). It also agrees with (Al-Tufaili & Nima., 2020) which recorded an increase in the MDA level for the infected group, reaching 12.493 ± 2.658 micromol/L for those infected with the amoeba parasite, compared to the control group, which reached 6.13664 ± 5.535 micromol/L.

The increased serum MDA levels due to lipid peroxidation in Entamoeba hisolytica patients is evidence of the deleterious effect on the host. Total carbohydrate protein is the most widely used biomarker for oxidative protein damage and shows the damage caused by many forms of ROS (Al-Tufaili& Nima.,2020).

The therapeutic effect of gold nanoparticles in the treatment complexes is due to their ability to scavenge oxygen species inside the body, inhibit the action of oxygen species and thus reduce the state of oxidative stress. The presence of oxygen species in the body's cells may increase the process of lipid peroxidation and thus increase MDA (Al-Badri, 2023).

3-3:Level of concentration of aminotransferase enzymes (AST and ALT)

The results showed a difference in the concentration rate of liver enzymes AST and ALT in laboratory rats infected with dysentery amoeba and treated with concentrations of 3.75 and 5 cm3 of gold nanoparticles, as an increase in the concentration rate of AST was observed for the positive control group, which reached 47.33 IU, compared to the negative control group, which reached 40.00 IU. An increase in the concentration rate of AST was also observed when using gold nanoparticles at a concentration of 3.75 and 5 cm3 for the infected groups.

It was also noted that there was an increase in the ALT concentration rate for the positive control group, which reached 34.67 IU, compared to the negative control group, which reached 23.67 IU. It was also noted that there was a decrease in the ALT concentration rate when using gold nanoparticles at a concentration of 3.75 and 5 cm3 for the infected groups (Table 3-3).

The results of our current study are consistent with (Hama Hasan et al., 2020), whose results showed a significant increase in AST and ALT levels (P < 0.05) in mice infected with the histolytic amoeba parasite compared to the control group, and also differ from (Sabeeh & Khalaf., 2022), whose results showed an increase in AST and ALT levels, as AST reached (22.84 +/- 7.83) in patients compared to (55.36 +/- 16.8) in the control group, and the ALT level was (20.4 +/- 9.37) in patients compared to (47 +/- 20.1) in the control group. It differs from ((Zangana & Erdeni ., 2020 in Tikrit, which showed a decrease in the AST concentration level for the group infected with the amoeba parasite, which reached 16.97 international units, compared to the healthy group, which reached 30.00 international units, and a decrease in the ALT concentration level for the group infected with the amoeba parasite, which reached 13.44 international units, compared to the healthy group, which reached 28.20 international units.

Table (**3**-3): Average concentrations of AST and ALT enzymes in laboratory rats infected with dysentery amoeba and treated with different concentrations of gold nanoparticles

Groups / **Test**	**Mean ± S.D**	
	ALT(GPT) **IU/L**	**AST(GOT) IU/L**
Negative control	23.00±5.30	**40.00±9.9**
Positive control	34.67±3.51	**47.33±3.06**
Gold nanoparticles 3.75 cm3	17.00±2.36	**50.80±8.35**
Gold nanoparticles 5 cm3	14.80±1.44	**42.00±7.49**
Statistical analysis	F-Value = 2.64* P-Value = 0.053	**F-Value = 2.99*** **P-Value = 0.049**

The mark * indicates the presence of significant differences at the level of P ≤ 0.05**.**

The reason for the increase is that these enzymes are mostly secreted and remain in the liver. However, when the liver is injured, these enzymes flow into the bloodstream. These enzymes are mostly found inside liver cells and to a lesser extent in muscle cells. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, which raises the levels of AST and ALT in the blood and indicates liver disease. The increase in AST and ALT is a result of the severe deterioration of liver tissue that occurs during the migration of the parasites (Kurdi & Mohammed, 2022). The increase in AST and ALT in rats infected and treated with gold nanoparticles can cause an inflammatory response in the body. If gold particles cause irritation or inflammation in tissues, including the liver, this may lead to increased levels of AST and ALT. Because gold nanoparticles can lead to direct toxic effects on the liver, causing damage to liver cells and an increase in AST and ALT enzymes.

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