Open Access
J. Glob. Innov. Agric. Sci., 2024, 12(4):1123-1129
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/24.1202
http://www.jgiass.com

Evaluating the Efficacy of *Artemisia annua* L-derived Artemisinin in Alleviating Visceral Leishmaniasis in Animal Models

Zahraa Ahmed Ali* and Hadeel Abdulatif Majeed

Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
*Corresponding author s e-mail: zahraaahmed.mc.s.zoo.2020@uomustansiriyah.edu.iq

The medicinal potential of *Artemisia annua L*., commonly known as sweet wormwood, has garnered significant attention due to its extraction of ART (Artemisinin ART drug) as a potent anti-malarial, antiviral, anti-inflammatory, and other therapeutic properties. With the urgency to address the neglected issue of VL in Iraq and the limitations of conventional antileishmanial therapies, The current study included two aspects: the first aspect *in vitro* focuses on exploring alternative treatments. Through a comprehensive *in vitro* assessment, we prepared ART extracts in varying concentrations (5, 10, 20, and 30 mg/ml) from *Artemisia annua L* And comparing its effect with pentostam (the traditional medicine used to treat leishmaniasis) at two different times: 24 and 48 hours. and examined their impact on the viability of visceral *Leishmania* parasite promastigote using the MTT assay, it turned out that the percentage of viability of the parasite in its promastigote stage decreased with increasing ART concentration, it also has an effect on parasite growth, as parasite growth decreases significantly with time and increasing concentration. The second aspects *in vivo*: investigation specifically focused on the effects of a 20 mg/ml concentration of ART drug on the livers of infected mice, revealing notable changes over a 21-day period. Our findings demonstrate the potential of the 20 mg/ml ART treatment, revealing distinct improvements in liver pathology over the course of treatment, emphasizing the promise of this alternative approach in combating VL.
Keywords: Artemisinin, *leishmania donovani*, parasite growth, visceral leishmaniasis, wormwood.

INTRODUCTION

Sweet wormwood an aromatic perennial herb belongs to the *Artemisia* genus. known plants that are used as traditional medicines to treat viral and inflammatory disorders because of their essential oils and powerful chemical components (Najm *et al.*, 2021; Uwah *et al.*, 2022).its original homeland is Asia, Thousands of years ago, the Chinese isolated and separated Artemisinin ART drug, ART is safe, non-toxic and well tolerated as a first-line treatment anti – malarial medication, as well as Antiviral, anti-inflammatory, allergenic, antifibrotic, antiarrhythmic, immunomodulatory ARTs and their derivatives have been implicated in autoimmune diseases (Xu *et al.*, 2020).Visceral leishmaniasis (VL) is one of the most dangerous types of leishmaniasis if it is not treated in time, An estimated 350 million individuals are at risk of contracting leishmaniasis (WHO. 2020). it is transmitted by a female sandfly genus (*Phlebotomine*) (Ibrra-meneses *et al.*,2022). the main cause of this disease is the parasite *Leishmania donovani*, it is important that VL was first discovered a very long time ago, VL has long been a public health concern in South-West Asia and the Arab World, At the middle of the eighteenth century, reports from Africa and India refer to the condition now known as visceral leishmaniasis as kal-azar, or black fever (Alves *et al.*, 2018). The groups most susceptible to this disease are children between the age 1- 4 year, the elderly, and those with weak immunity (Dias-Lopes *et al.*, 2021) A number of blood symptoms can be caused by Leishmaniasis, including a chronic course, enlarged lymph nodes, liver and spleen, fever, Anemia on by ongoing inflammation, weakness and exhaustion, weight loss, diarrhea and anorexia, And other symptoms (Colomba *et al.*, 2022). pentavalent antimonial, used as the first drug for over 70 years, it is still used in many parts of the world as sodium stibogluconate (Pentostam) or meglumine antimonate (Glucantime) (Croft and Olliaro, 2011). Additionally, the type of *Leishmania* most commonly infecting humans has been shown to be resistant to chemotherapy (Das *et al.*, 2013). New drugs have therefore been developed to treat leishmaniasis important. Aim of current study was evaluate the antiparasitic effect of Artemisinin extracted from a plant *Artemisia annua L* and

Ali, Z.A. and H.A. Majeed. 2024. Evaluating the Efficacy of *Artemisia annua* L-derived Artemisinin in Alleviating Visceral Leishmaniasis in Animal Models. Journal of Global Innovations in Agricultural Sciences 12:1123-1129.
[Received 11 Nov 2023; Accepted 10 Nov 2024; Published 17 Nov 2024]

consider is the traditional medicine for the malaria parasite. Therefore, the effect of ART drug examined on *L. donovani* promastigotes was investigated. investigation the effect of ART drug extracted from *Artemisia annua L.* on the liver of mice infected with *L. donovani* and compare the effect of ART drug with other groups.

MATERIALS AND METHODS

***Artemisinin extracted (drug)*:** It is a medicine extracted from the leaves of the *Artemisia annua L.* plant in its pure form, packed in capsule form, manufactured in the USA.

Figure 1. leaves of the *Artemisia annua L.* plant.

***Preparation of different concentrations of the artemisinin drug*:** Four different concentrations of ART drug were prepared (5, 10, 20 and 30 mg/ml) by opening the capsule and weighing an amount of its powder with a sensitive balance, then dissolving it in the injection water to obtain the required concentration according to the equation.

Concentration = Weight/Volume = mg/ml

***Parasite strain and culture*:** *Leishmania donovani* cultured on RPMI-1640 medium was obtained from the College of Science / University of Baghdad, it was cultured and maintained by serial passage in Novy-MacNeal-Nicolle (NNN media) each 8 days incubated at 27°C.

***Measured the effect of artemisinin drug on the Leishmania donovani promastigotes viability in vitro by using Methylthiazol Tetrazolium assay (colorimetric MTT assay)*:** The effect of ART drug on *L. donovani* promastigotes was measured by using the MMT[3-(4,5-dimethyl-thiazoly-2-yl)-2,5-diphynel tetrazolium bromide]. where ART drug was used in various concentrations (5, 10, 20 and 30 mg/ml), and 0.041 mg/ml from pentostam drug for comparison. SHE medium was also used as an alternative to RPMI-1640 medium in incubation (Ali *et al.,* 2009). The experiment is carried out using an ELISA reader device (huma reader HS) Made in Germany, 96-hole MTT kits, the experiment's reagent, and DMSO dye.

***Animal Grouping*:** From the National Centre for Drug Control and Research / MoH, 80 albino mice weighing 20-28 gm and aged 8-12 weeks were procured. The mice were maintained under standard conditions in the biology department of Mustansiriayah University's College of Science's in animal house. Ten mice from eighty left healthy are considered a negative control (G1), while seventy, *L. donovani* (promastigotes) were intraperitoneally injected (1×10^7 parasites/ml) into mice to infect them. After14 days, seven infected mice were dissection to make sure of the infection was occurring. The liver was removed then prepared impression smears on a slide, stained by giemsa stain and examined under the oil lens of light microscope to confirm the presence of amastigote inside the cells (phagocytes). The infected mice were then split into three groups, each with 21 animals. After that, each group received an inoculation as follows:

- ***Group 1 (G1)*:** Ingested orally by stomach tube (0.1 ml/ day) normal saline for 21 days, considered as negative control group, (healthy group).
- ***Group 2 (G2)*:** ingested orally by stomach tube (0.1 mL/ day) normal saline for 21 days, considered a positive control group, (Infected group).
- ***Group 3 (G3)*:** ingested orally by stomach tube, ART drug of (20 mg/ ml) concentration (0.1mL/day), considered as ART drug treated group, (infected).
- ***Group 4 (G4)*:** injected with 0.1mL/day from Pentostam drug (0.041 mg/ml) by intramuscularly considers as pentostam treated group, (infected).

***Histological Study of Mice liver*:** After 7th and 21th days the mice dissection, and the liver was removed and fixed in 10% formalin. Then processed by washing, dehydration process, clearing, infiltration, embedding, trimming, sectioning and finally staining with hematoxylin and eosin for studying histological changes (Mohamed *et al.,* 2019).

RESULTS

***Effect of Artemisinin Drug on Survival of Leishmania donovani Promastigtes Using Methylthiazol Tetrazolium assay*:** The antiparasitic effect of four different concentrations (5, 10, 20 and 30) mg/ml of ART drug was evaluated using the MTT assay which applied on *L. donovani* promastigotes. According to the findings, there were statistically significant differences between the various dosages of the ART drug and the eradication of the *L. donovani* parasite, particularly at a dosage of 30mg/ml which exhibit the effect similar to that of pentostam. These outcomes were achieved in comparison to Pentostam, a medication that is frequently used to treat this parasite. It is clear from the MTT assay, that the ART drug is fatal to the *L. donovani* parasite, and the viability rate of promastigotes decreases with the increase in the concentration of the ART drug.

Table 1. The viability percentage of *Leishmania donovani* parasites after exposure to different concentrations of Artemisinin and pentostam by MTT assay at two different times.

P Value	Control positive	Pentostam drug 0.041mg/ml	ART drug Concentration mg/ml viable cell %				Time duration
			30mg/ml	20mg/ml	10mg/ml	5mg/ml	
0.001	100%	70%	62%	71%	73%	75%	24h
0.001	100%	55%	50%	57%	66%	69%	48h
P value		0.01	0.01	0.01	0.01	0.01	

P value = between tested groups and pentostam; ART = Artemisinin

***Diagnosis of Leishmania donovani using Giemsa stain*:** Two weeks after infection of mice with *L. donovani* parasite, a number of mice were dissected, their livers were taken, smears were made and stained with Giemsa stain, to detect the presence of amastigotes (Leishman bodies) inside the liver, the slide was examined with a light microscope at X100 power at oil immersion objective lens. It is worth noting that this step comes before dividing the mice and using different treatments, as shown in figure (2).

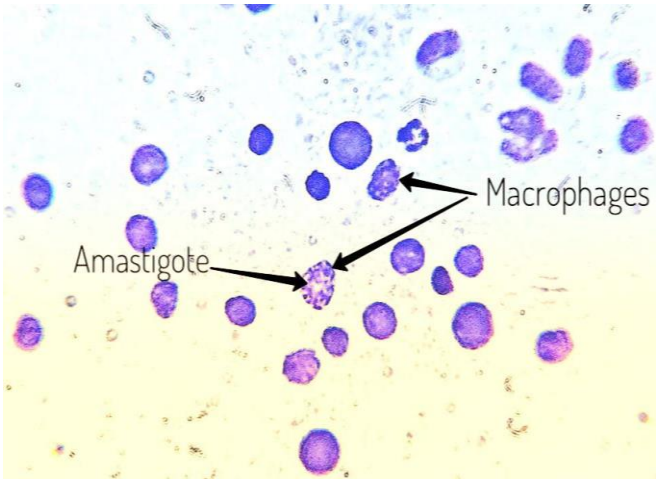

Figure 2. Diagnosis of *Leishmania donovani* using Giemsa stain

***The effect of Artemisinin on liver tissue of mice and its comparison with the other groups*:** Mice were dissected at the 7th and 21th day from all the groups in order to compare the histological differences that occurred between the four groups, so the liver was removed and slide sections of the liver were prepared, the hematoxylin and eosin stain (H & E stain) was used to examine these after *L.donovani* promastigotes infected mice. these mice were split into three groups; one group received treatment with pentostam, another was given ART changes. After the seventh day, the infected mice (control positive) group revealed histological changes in the liver compared to the uninfected mice (control negative) group, which displayed normal appearance of the central vein , hepatic cords, sinusoids, and hepatocyte figure (3) ,while the liver section for control positive group after 7 days figure (4) , revealed moderate cellular swelling and necrosis of hepatocytes with glycogen degradation , moderate sinusoidal dilation, and the magnified sections revealed mild infiltration of leukocytes into the central veins and sinusoids , on the other hand, after one week from treated the mice with ART drug figures (5), the liver showed moderate dilation with congestion of the central vein, marked congestion with dilation of the portal vein and intravascular hemolysis, necrosis of hepatocyte and showed normal sinusoid , figure (6) showed congestion of central vein, with simple focal necrosis, mild cellular swelling of hepatocyte and glycogen degradation, the magnification of the figure revealed normal sinusoid and little infiltration of lymphocytes, this figure belong to liver of mice treated with pentostam for 7 day. In addition, after 21day, the damage in the control positive group progressed to severe state, with numerous localized necrosis, sinusoidal dilatation, and infiltration of inflammatory cells along with disorganization of the hepatic cords, figure (7). Figure (8) observed the liver treated with ART drug for 21 days, it exhibited mild dilation of sinusoid, normal binuclear hepatocytes, and cytoplasmic vacuolation. Also, after 21 days of treated mice with pentostam, the liver showed normal central vein, normal hepatocytes with binuclear, normal sinusoid and little infiltration of lymphocytes, figure (9).

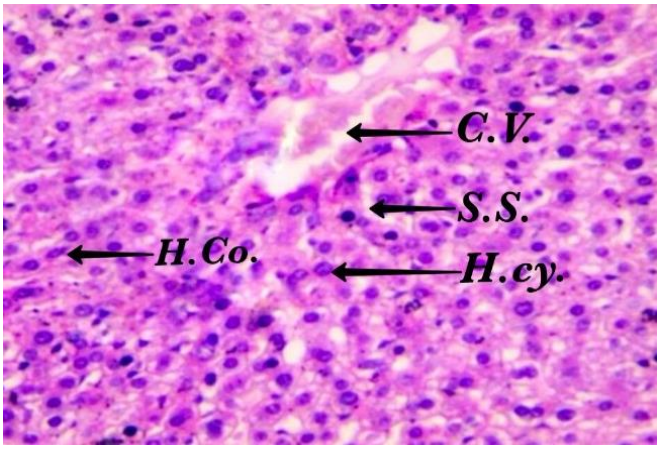

Figure 3. cross section of liver in mice (control negative) group showing normal histological structure of hepatic tissue. (C.V.) central vein, (H.cy.) hepatocyte, (H. Co.) hepatocord, (S.S.) sinusoid. (H & E stain, 20 X).

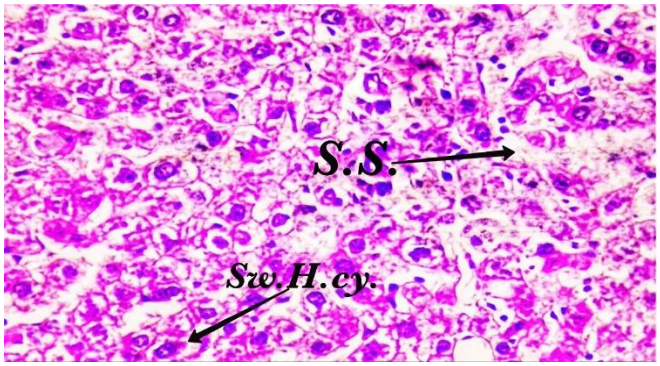

Figure 4. Cross section of liver in mice infected with *leishmania donovani* after one weeks, showing: moderate dilation sinusoid (S.S.), swelling of hepatocyte (Sw.H.cy.) with glycogen degradation, (H & E stain, 40 X).

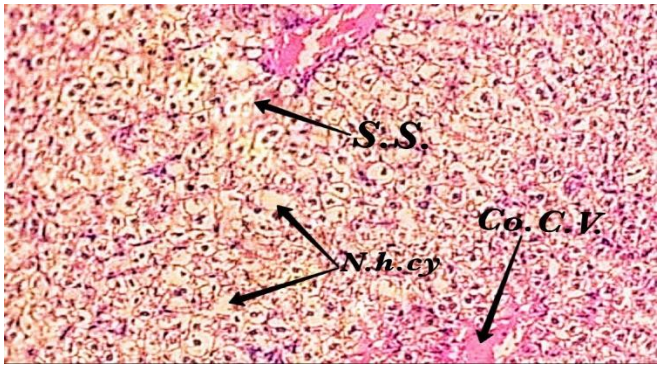

Figure 5. Cross section of liver in mice treated with 20 mg/ml of Artemisinin drug for seven days, showing: moderate dilation with congestion of central vein (Co. C.V.), necrosis of hepatocyte (N.H.cy), normal sinusoid (S.S.), (H& E stain, 10X).

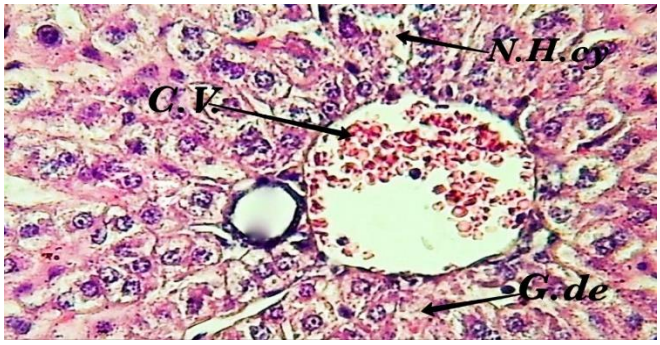

Figure 6. Cross section of liver in mice treated with pentostam drug for seven days, showing: congestion of central vein (C.V.), glycogen degradation (G.de) and simple necrosis of hepatocyte (N.H.cy), (H & E stain, 40 X).

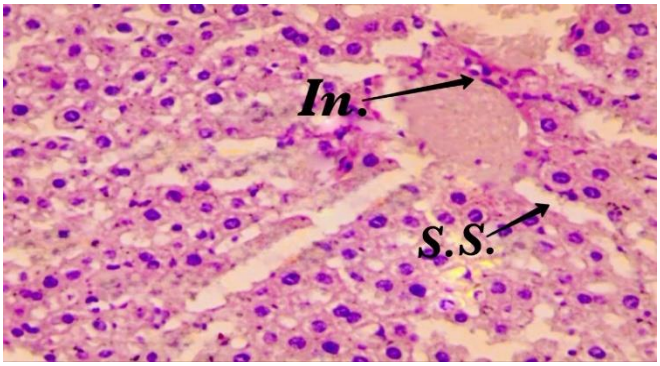

Figure 7. Cross section of liver in mice infected with *leishmania donovani* after three weeks, showing: sever damage with dilation sinusoid (S.S.), infiltration of inflammatory cell (In.) and with disarrangement of hepatic cord, (H & E stain, 40X).

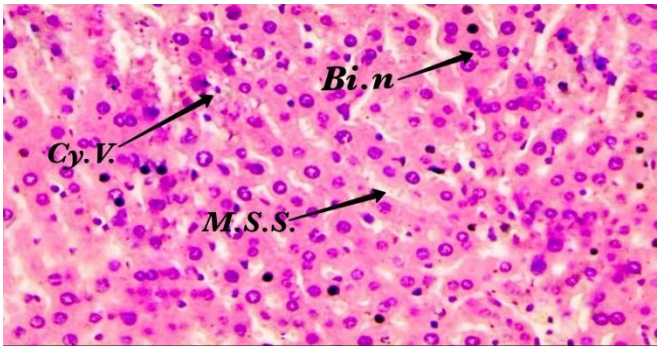

Figure 8. Cross section of liver in mice treated with 20 mg/ml of Artemisinin drug for 21 days, showing: normal histological structure appearance of hepatic tissue with mild sinusoidal dilation (M.S.S.), binuclear cell (Bi. n) and cytoplasmic vacuolation (Cy. V.), (H & E stain, 20 X).

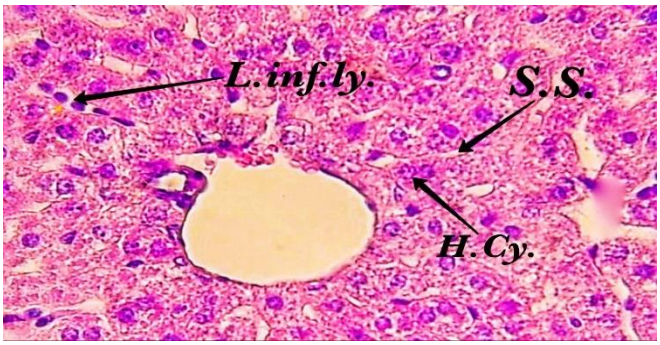

Figure 9. Cross section of liver in mice treated with pentostam drug for three weeks, showing: normal sinusoid (S.S.), normal hepatocytes (H.cy.), little infiltration of lymphocytes (L. inf. Ly.), (H & E stain, 40 X).

DISCUSSION

***Effect of Artemisinin Drug on Survival of Leishmania donovani Promastigtes Using MTT Assay*:** Due to their toxicity and need for hospitalization for administration, the chemotherapy anti-leishmaniasis drugs considered to be the first line of treatment sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime) have been found to cause side effects like pancreatitis, cardiotoxicity, and nephrotoxicity (Taher *et al*., 2015). An earlier study by Sen *et al*. (2010) looked into whether the same processes underlie Artemisinin's anti-malarial action in *L. donovani* promastigotes. This was done since iron-mediated production of free radicals supports Artemisinin's anti-malarial activity. By producing more free radicals and producing fewer non-protein thiols, ARTs drug successfully altered the redox potential (Sen *et al*., 2010) It has been discovered that the antimalarial medicine artemisinin and its derivatives are also effective against non-malarial parasites like *L. donovani*. ARTs drug and its derivatives with a high safety index have been shown to have antileishmanial action in several *in vitro* and *in vivo* experiments (Ghaffarifar *et al.,* 2015). Regarding the mode of action, it has been observed that artemisinin and its derivatives cause cell cycle arrest, DNA fragmentation, and externalization of phosphatidylserine in *Leishmania* promastigotes, leading to programmed cell death (Ghosh *et al.,* 2020)

***Diagnosis of Leishmania donovani using Giemsa stain*:**The results of the Giemsa-stained liver stain showed the presence of the *L. donovani* parasite in its amastigote stage inside macrophage cells (Abd *et al.,* 2018).

***The effect of Artemisinin on liver tissue of mice and its comparison with the other groups*:** Understanding the ART drug effect on the liver of VL-infected mice and the histological alterations that take place in all cases is crucial to the current study. According to a study in 2019, liver involvement in visceral leishmaniasis might manifest as localized and piecemeal necrosis, intracellular amastigotes, and chronic granulomatous hepatitis. However, liver biopsy specimens showed a widespread hepatitis process and a necro-inflammatory pattern without granulomas (Martinez de Narvajas *et al.,* 2019). Another recent study in 2021, demonstrated that *Leishmania visceral* infection causes pathogenic changes in the reticuloendothelial system as well as mild pathological changes in the mice's livers, including the development of granulomas and centrilobular necrosis and liver disorganization and hypocellularity, hepatic sinusoidal dilation and congestion, and mild hepatic central vein congestion (Kadhem *et al.,* 2021). The liver is an important organ affected by VL, as the parasite can be diagnosed during the first weeks of infection. Infection occurs after the parasite has spread and damaged the reticuloendothelial system. An asymptomatic infection progressing to systemic disease with hepatomegaly in VL patients is initially possible (Abreu-Silva *et al*., 2004). Claim that the accumulation of amastigotes in the liver and spleen, which also results in hyperplasia in the cells of the endothelial reticulum, is the cause of hepatosplenomegaly, which manifests 30 days after infection and increases macrophage protection as a defense against parasites. Kupffer cells were found to contain *L.* amastigotes in all of the patients. Hepatic histological alterations were linked to ischemic necrosis, macro vesicular steatosis, portal inflammation, and piecemeal necrosis (Artan *et al.,* 2006). As the disease advances, VL patients typically have a fever, weight loss, and enlargement of the liver and spleen due to the invasion of parasites in the reticuloendothelial cells. In accordance with previous findings of the positive control group, leishmaniasis is characterized by significant lesions, including numerous localized necrosis, sinusoidal dilatation, and inflammatory cell infiltration with a disorder of the hepatic cords. These results are supported by previous study in 2012, which found that there was severe scattered hepatic necrosis and an infiltration of lymphocytes and macrophages that consumed leishmanial particles (Al-Harmni *et al.,* 2012). On the other hand, artemisinin had strong anti-cancer properties both *in vitro* and *in vivo*. Additionally, research has indicated that artemisinin (ART) may have anti-inflammatory and anti-liver fibrosis benefits. And has been evidence of artemisinin's potential role in the control of liver inflammatory reactions. Effectively, herbal items are used all over the world to treat hepatic ailments, and one of these medications is ART, which is used to cure and prevent liver diseases (Amat *et al.,* 2010). A recent study in 2018, observed the central veins, hepatic lobules, and hepatic sinusoids of the control and artemisinin groups were all present and functioning normally. The liver injury group's (positive control) hepatic tissues had significant hepatotoxicity, many irregularly distributed zones of necrosis, and a lack of cellular and tissue architecture information. Another recent study in 2021, studied liver infections, such as VL group, co-treated with artemisinin revealed regeneration of hepatic lesions with primarily normal hepatic tissue. Additionally, earlier studies have demonstrated that ART drug and its derivatives can reduce liver fibrosis, liver infections, such as VL group, co-treated with Artemisinin revealed regeneration of hepatic lesions with primarily normal hepatic tissue. Additionally, earlier studies have demonstrated that ART drug and its derivatives can reduce liver fibrosis (Xiong and Huang, 2021). Models concerning how ART drug might operate physiologically were mostly derived from knowledge from in vitro medicinal chemistry investigations due to a lack of fundamental understanding of the molecular, histological, and subcellular features of ART drug in *vivo*. Many studies have focused on the chemical interactions of ART drug with iron, either in the nonheme or heme form (Zhang and Gerhard, 2008). The results of a previous study in 2010, demonstrated that the iron-Artemisinin adducts seen in experimental visceral leishmaniasis were produced by the Artemisinin

action on intracellular Leishmania - infected mice, which eradicated the intracellular amastigotes (Sen *et al.*, 2010). On the other hand, mice were used to test the anti-leishmanial efficacy of pentostam. the mice infected with *L. donovani* received the treatment on the seventh and eighth days after infection. Both treated and control animals showed considerably reduced parasite levels in the liver (99% suppression) on the two weeks following infection, but it had no effect on parasite counts in the spleen or bone marrow. The carrier forms of the medication were therefore far more effective than the free medicine at reducing parasite burdens in the liver (Carter *et al.*, 1988).

***Conclusion*:** According to the obtained results of this study, the following conclusions are reached:

1. The results indicate the ART drug had inhibitory effect on viability of *L. donovani* promastigotes parasite.
2. Concentration 20 mg/ml of ART drug had a good effect on the liver tissue.
3. Artemisinin drug has a therapeutic effect approximately similar to that of pentostam, which is considered the traditional drug for treating visceral leishmaniasis

***Acknowledgement*:** The authors would like to thank:

- Al-Mustansiriyah University, Iraq (www.uomustansiriyah.edu.iq) for supporting the present work.
- University of Baghdad, Iraq (http://uobaghdad.edu.iq) for providing pure isolates of the parasite *Leishmania donovani.*

***Ethical statement*:** Informed consent according to the Declaration of Helsinki was obtained from ethics committee College of Science, Al-Mustansiriyah university. Ref. (BCSMU/0822/0016Z), Appendix-(1).

***Funding*:** None.

***Informed consent*:** N/A

***Conflict of interest*:** authors have not any conflict of interest.

***Author contribution*:** Zahraa Ahmed Ali and Hadeel Abdulatif Majeed are participated in all process of study.

***SDGs addressed*:** Good Health and Well-being, Zero Hunger and Clean Water and Sanitation.

REFERENCES

Abd, A.N., S.T. Mohammed, M.F. Al–Marjani, J.A.S. Salman, S.D. Salman and N.F. Habubi. 2018. Antiparasitic Effect of CdO NPS Synthesized by Simple Chemical Method SCM on Leishmania donovani in vivo and in vitroherbicide glyphosate. Mesopotemia Environmental journal 2018:97-106.

Abreu-Silva, A.L., K.S. Calabrese, R.A. Mortara, R.C. Tedesco, F.O. Cardoso and L.O. Carvalho. 2004. Extracellular matrix alterations in experimental murine Leishmania amazonensis infection. Parasitology 128:385-390.

Ali, E.N., S.T. Mohammed, H.A. Ajah and R. Jamal. 2009. Preparation a New SHE-Medium Replacement of RPMI1640-Medium using Oral Rehydration Solution. Journal of Global pharma Technology, 12:196-200.

Alves, F., G. Bilbe, S. Blesson, V. Goyal, S. Monnerat and C. Mowbray. 2018. Recent development of visceral leishmaniasis treatments: successes, pitfalls, and perspectives. Clinical microbiology reviews 31:10-1128.

Al-Harmni, K.I., Z.I. Rahemo and H.I. Al-Khan. 2012. The effect of two fruit extracts and drugs on the liver of albino mice with induced leishmaniasis. Trends in Parasitology Research 1:16- 26.

Amat, N., H. Upur and B. Blažeković. 2010. In vivo hepatoprotective activity of the aqueous extract of Artemisia absinthium L. against chemically and immunologically induced liver injuries in mice. Journal of ethnopharmacology 13:478-484

Artan, R., A. Yilmaz, M. Akçam and N.H. Aksoy. 2006. Liver biopsy in the diagnosis of visceral leishmaniasis. Journal of gastroenterology and hepatology 21:299-302.

Carter, K.C., A.J. Baillie, J. Alexander and T.F. Dolan. 1988. The therapeutic effect of sodium stibogluconate in BALB/c mice infected with Leishmania donovani is organ-dependent. Journal of pharmacy and pharmacology 40:370-373.

Colomba, C., C. Guccione, R. Rubino, M. Scalisi, A. Condemi and S. Bagarello. 2022. Third Case of Visceral Leishmaniasis in COVID-19: Mini Review Article. Pathogens 11:913.

Croft, S.L. and P. Olliaro. 2011. Leishmaniasis chemotherapy—challenges and opportunities. Clinical microbiology and infection 17:1478-1483.

Das, S., P. Roy, S. Mondal, T. Bera and A. Mukherjee. 2013. One pot synthesis of gold nanoparticles and application in chemotherapy of wild and resistant type visceral leishmaniasis. Colloids and Surfaces B: Biointerfaces 107:27-34.

Dias-Lopes, G., A. Zabala-Peñafiel, B.C. de Albuquerque-Melo, F. Souza-Silva, L.M. do Canto and L. Cysne-Finkelstein. 2021. Axenic amastigotes of Leishmania species as a suitable model for in vitro studies. Acta tropica, 220:105956.

Ghaffarifar, F., F.E. Heydari, A. Dalimi, Z.M. Hassan, M. Delavari and H. Mikaeiloo. 2015. Evaluation of apoptotic and antileishmanial activities of Artemisinin on promastigotes and BALB/C mice infected with Leishmania major. Iran. Journal of Parasitology 10:258.

Ghosh, S., A. Verma, V. Kumar, D. Pradhan, A. Selvapandiyan and P. Salotra. 2020. Genomic and

transcriptomic analysis for identification of genes and interlinked pathways mediating artemisinin resistance in Leishmania donovani. Genes 11:1362.

Ibarra-Meneses, A.V., A. Corbeil, V. Wagner, C. Onwuchekwa, and C. Fernandez-Prada. 2022. Identification of asymptomatic Leishmania infections: a scoping review. Parasites and vectors. 15:1-22.

Kadhem, Z.A., A.A. Aakool and S. A. Faraj. 2021. Pathological Changes of Visceral Leishmaniasis on Liver and Spleen in Experimentally Infected BALB/C Mice. Annals of the Romanian Society for Cell Biology 2021:5078-5086.

Martinez de Narvajas, I., A. Díaz, O. Bassegoda, A. Carpio, C. Fuster and M.E. Valls. 2019. Acute liver failure due to visceral leishmaniasis in Barcelona: a case report. BMC infectious diseases 19:1-5.

Mohamed, S.T., H.H. Ghadi, S.B. Kamal, N. Mohammed and H.A. Aja. 2019. Effect of Fusarium graminarum silver nanoparticles on liver tissue of infected mice of visceral leishmaniasis. Journal of Pharmaceutical Sciences and Research 11:886-891.

Najm, M., R Hadighi, M. Heidari-Kharaji, M. Alipour, M. Hajizadeh, R. Rafiei-Sefiddashti and A. Badirzadeh, 2021. Anti-Leishmanial Activity of Artemisia persica, A. spicigera, and A. fragrance against Leishmania major. Iranian Journal of Parasitology 16:464.

Sen, R., P. Saha, A. Sarkar, S. Ganguly and M. Chatterjee. 2010. Iron enhances generation of free radicals by Artemisinin causing a caspase-independent, apoptotic death in Leishmania donovani promastigotes. Free radical research 44:1289-1295.

Taher, J.H., Y.M. AL-Mulla Hummadi, N.M. Al-Bashir and A.S. Al-Araji. 2015. Lactate dehydrogenase (LD), alkaline phosphatase (ALP) iso enzymatic patterns in Iraqi children with visceral leishmaniasis before and after treatment with stibogluconate. Journal of Parasitic Diseases 40:277-284.

Uwah, A.F., B.O. Effiong, J.S. Obot, I.J. Obioku and A.E. Udoh. 2022. Therapeutic potential of concurrent administration of Hippocratea africana and Eremomastax speciosa in the treatment of Plasmodium berghei infected mice. Ibom Medical Journal 15:166-174.

World Health Organization. 2020. Control of the leishmaniasis. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. Geneva (Switzerland): World Health Organization.

Xiong, Y. and J. Huang. 2021. Anti-malarial drug: the emerging role of artemisinin and its derivatives in liver disease treatment. Chinese Medicine 16:80.

Xu, C., H. Zhang, L. Mu and X. Yang. 2020. Artemisinin as anticancer drugs: novel therapeutic approaches, molecular mechanisms, and clinical trials. Frontiers in Pharmacology 11:529881.

Zhang, S. and G.S. Gerhard. 2008. Heme activates Artemisinin more efficiently than hemin, inorganic iron, or hemoglobin. Bioorganic and Medicinal Chemistry 16:7853-7861.