



## **Fertility study of hydatid cysts isolated from slaughtered camels and its relationship to affected organs in Dhi Qar Governorate in Iraq**

**Abdul-Jaleel Aziz Karim Alqaraghli**

### **Abstract**

Hydatid cyst disease (HCD) is an endemic zoonotic disease caused by the larval stage of the parasite *Echinococcus granulosus*.

Forty samples of hydatid cyst of camels were collected from Nasiriyah, slaughter and butchers shops, during the period from January 2021 to April 2021. Protoscolices and the germinal layer (GL) were isolated from those samples.

The results showed that the camel infection rate was 8.33% out of 120 examined animals, the infection was concentrated in the liver (50%), lungs (30%) and spleen (20%). The majority of cysts were calcified with a percentage of 54.55%, while the fertile were predominant over the sterile, which amounted to 27.27% and 18.18% respectively. It also appeared that the infection was more in females (70%) than in males (30%).

**Keywords:** *Echinococcus granulosus*, fertility, camels

## Introduction

Hydatid cyst disease (HCD) or the so-called cystic echinococcosis (CE) is a serious and important zoonotic disease [1], an endemic disease that is difficult to treat [2], this disease is caused by infection of the intermediate host (sheep Cows and other livestock as well as humans) in the larval stage of the *E. granulosus* parasite of the Taeniidae family [3].

CE has become a major health concern [2], which is further complicated by the fact that no systematic surveillance and control program for CE has been established in Iraq [4,5].

The infection of Iraqi camels with HCD has negative effects on an important aspect of livestock, and the high percentage of camels affected with hydatid cyst confirms the contribution of camels to spreading the infection as an important intermediate host in the life cycle of the parasite *E. granulosus* to both humans and animals alike. [6].

Attributes [7,8] the prevalence of (HCD) in the Middle East to several factors, the most important of which are the rural-nomadic lifestyle, the large number of farm animals, and the use of herding dogs. Some of the feeding habits include the high consumption of fresh vegetables, the spread of unorganized and poor quality massacres, the widespread slaughter of animals in homes, especially on religious and social occasions, the lack of people's awareness of the disease and its transmission methods, the multiplicity of medium host types, and the high numbers of stray dogs.

## Material and methods

### Sample collection

Camel samples were obtained from the Nasiriyah butchery and butchers shops. Hydatid cysts have been detected in the affected animal's organs. As for the presence of a white or yellowish-white bubble-like layer on the surface of the external organ, especially in the liver, it is very clear, but some cysts are located deep in the affected organ, like most lung cysts, in camels. Deep-seated cysts can be detected by palpating the suspected cysts and then carefully dissecting the tissues around this area to avoid a strong outflow of hydatid fluid. The hydatid fluid in fertile specimens tends to be yellowish-white; because it contains a large number of protoscolices. The fluid in some cysts of the liver and spleen tended to yellow, while in some sterile cysts it was pure white. These samples were then transferred to sterile containers in cooler cork boxes, and kept under refrigeration until each cyst was examined and prepared for the subsequent steps.

### Isolation of protoscolices and germinal layer

The method [7,9] was followed to sterilize the outer surface of the affected organ and the cysts it contains as a first step, using 70% ethanol alcohol to prevent contamination, then 10 or 5 ml syringes were used to puncture the cyst from the sides to withdraw the hydatid fluid from it. 95% of the liquid was withdrawn and placed in test tubes of 10 or 15 ml, one drop was placed on a glass slide and covered with the slide cover and examined by a light microscope with a power of 100X and 400X to confirm the presence of protoscolices, then a peripheral incision was made in the fibrous envelope (outer) to the cyst using a scalpel and tweezers, then the generated layer was pulled out with forceps and placed inside the preservation vials.

The subsequent steps were carried out according to the [8] method, where the tubes were placed in a centrifuge at 3000 rpm for 15 min., then the upper liquid was poured out when the time was up, and the sedimentary layer of protoscolices that could be observed under the tubes was kept, in case the cyst was fertile, the precipitate was washed by adding 10 ml of physiological saline. Repeat this step at the same speed and duration 3-5 times; To filter protoscolices.

A number of small pieces were taken from the GL and placed in test tubes; To perform the aforementioned washing process with protoscolices, all in the event that the cyst is sterile.

Hydatid cyst fertility was studied by direct microscopy of hydatid fluid under 400X for the presence of protoscolices and using eosin staining [10].

## Results

### Morphological study of livestock hydatid cysts

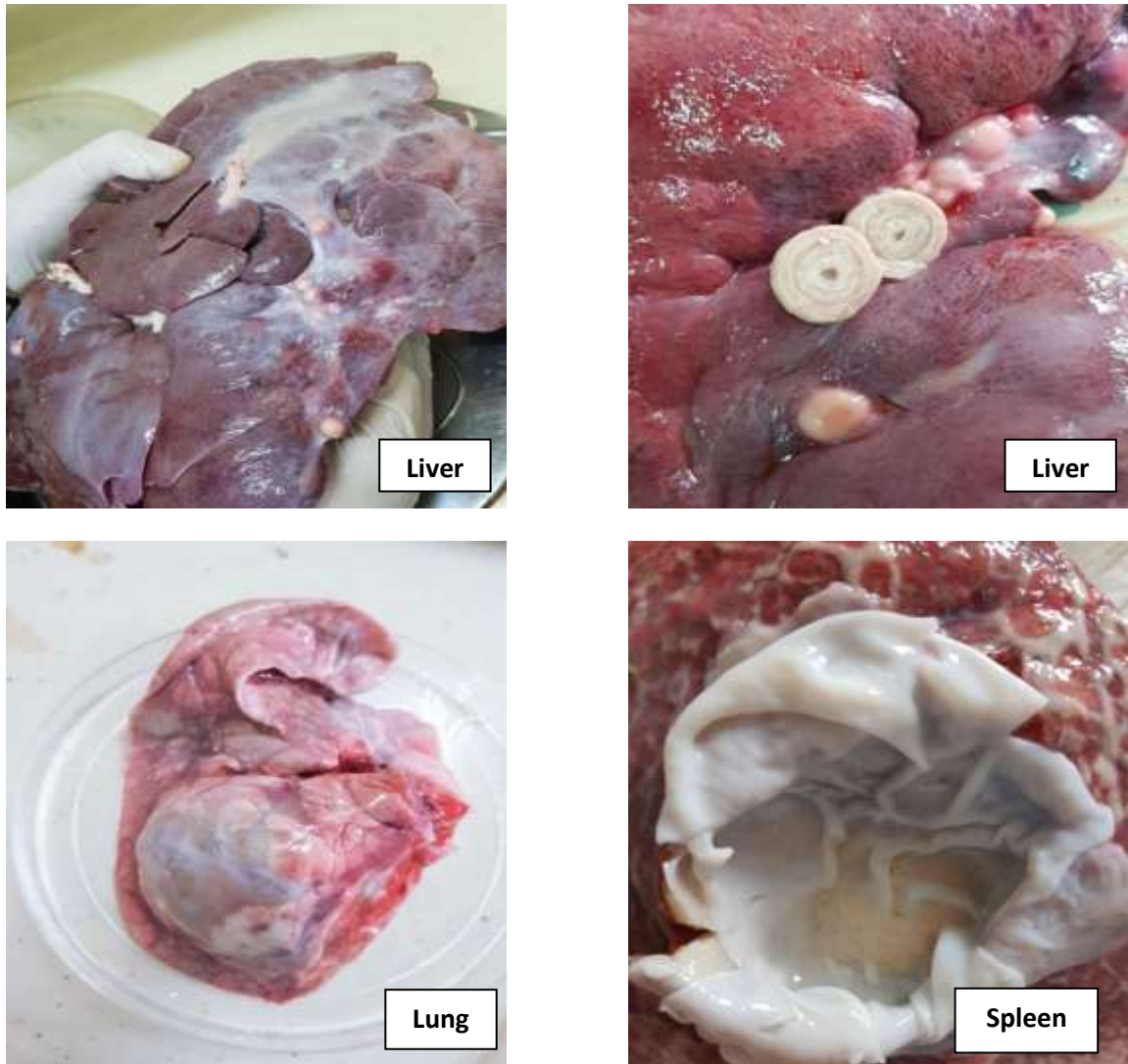
The percentage of infection in camels was 8.33%, as the number of examined camels was 120 animals, 10 of them were affected with (HCD), 40 hydatid cyst samples were collected. These cysts were found in the liver and lungs of camels. On the upper surface of the organ, it was clearly visible, and it was a fluid-filled sac or a hard sac in case it was calcified. As for most of the sacs, they were embedded within the tissue of the organ and were not clearly visible. They were identified through palpation using the fingers in the organ tissue, and the tissue around this area was carefully dissected to avoid strong fluid flow.

### Distribution of hydatid cysts according to affected organs

The results of the research showed that the incidence of hydatid cysts in camels was concentrated in the liver, lungs and spleen. The liver is the most affected organ, with a percentage of 50% of infection, the spleen 20% being the lowest, and 30% of the lungs, as shown in table (1) below.

**Table 1:** Percentage of hydatid cyst infections by affected organ

Affected organ	Number	Percentage (%)
Liver	5	50
Lungs	3	30
Spleen	2	20
Total	10	100

**Fig.1.** Liver, lung and spleen of camels infected with hydatid cysts.**Fertility of Hydatid cysts in camel**

The results of our research showed that the largest percentage of hydatid cysts in camels was calcified, as there were 18 calcified cysts with a percentage of 54.55% in each of the organs (liver, lungs and spleen).

for the fertile cysts, they were predominant over the sterile cysts, as the number of fertile cysts was 9 with a percentage of 27.27%, as they contained protoscolices and brood capsules inside their hydatid fluid. As for the sterile cysts, they were the least with a percentage of 18.18% sterile hydatid cysts and they are not It contains protoscolices within the hydatid fluid, as shown in Table (2) below.

**Table 2:** Type of hydatid cyst in affected camels

Cyst type	Number	Percentage (%)
Calcified	18	54.55
Fertile	9	27.27
Sterile	6	18.18
Total	33	100

Our results also showed that the infection in females was the most, reaching 70% with 7 infections, compared to males with 3 and 30%.

**Table 3:** The number of hydatid cysts by animal gender

Gender	Number	Percentage (%)
Females	7	70
Males	3	30
Total	10	100

## Discussion

The current study indicated that the liver is the organ most susceptible to HCD, and this agrees with most studies [11-18].

The current study agreed with the study of [19-25] in the central Euphrates regions of Iraq that liver injuries were the most, at 67.5% of the total, and lung injuries were 20%. It also agreed with [26,27] in Muthanna Governorate, where liver injuries were 84%. Of the total total and lung injuries by 14%. In a study conducted in the Kingdom of Saudi Arabia [28-33] stated that the liver and lung were the most affected visceral organs in all the animals examined, and the prevalence of liver injury was 100% in camels.

Our study did not agree with the study [34,35] in Libya, which found that the lungs were the most affected organ in camels, with 55.2%. As well as a study [36-39] in Iran, which showed that the percentage of lung injury was 90%, while the percentage of liver injury was 10% of the total number of injuries.

The cause of frequent liver infections with hydatid cysts may be due to the fact that the hexacanth embryos penetrate the intestinal mucosa and begin to migrate passively through the blood in the portal vein to reach the liver where one (or more) embryos begin to grow and develop into a hydatid cyst containing the cyst fluid. and protoscolices [1] Because of the large size of the liver, in most fetuses the liver is the first site of infection with hydatid cysts in the host body [16,40]. Some studies also mentioned that the Intermediate hosts lung is a prevalent site of infection with hydatid cysts, due to the large lymphatic vessels, which provide an opportunity for the fetus to reach the lymphatic capillaries, then move through the lymphatic vessels to the lungs before being transferred in the veins to the liver [15,41]. he results of our research showed that the largest percentage of hydatid cysts in camels was calcified with a percentage of 54.55%. As for fertile cysts, they were predominant over sterile cysts with a percentage of 27.27%, while sterile cysts were the least with a percentage of 18.18% sterile hydatid cysts. These results agreed with the study [12,42] in Iraq, where the proportion of fertile cysts in camels was 60%, which is higher than the proportion of sterile cysts, which reached 40% of the total, as well as with the study [20] where the proportion of fertile cysts in camels is 53.9%, which is The lungs are higher than the livers. The current study agreed that fertile sacs are the highest with a study [16] in Iran, where 20 udder sacs were examined and the number of fertile sacs was found 14 at a rate of 70%, while the number of calcified sacs was 6 at a rate of 30%.

The high percentage of calcified cysts may be due to the outdated infection of hydatid cysts and thus their calcification, as butchers usually slaughter old animals in these areas. Or the high ability of camels to endurance, fight disease and high immunity, which has not been proven in any research except that it is a result reached by everyone who studies this animal.

The difference in fertility between the different studies may be due to the difference in the environment surrounding the animal, whether it is a rural or nomadic environment full of dogs and other animals that are intermediate hosts.

As for the high percentage of calcified cysts, this may be due to the camel's high ability to bear, fight disease and high immunity, which has not been proven in any research except that it is a result reached by everyone who studies this animal, which has long been known as an animal of harsh arid desert conditions, endurance and adaptation to the environment.

The results of the current study showed that the incidence of hydatid cysts in females was higher than in males by 70% and 30%, respectively.

These results agreed with a study [21,43] in Dhi Qar, where it was found that the rate of infection of females is higher than that of males with (HCD). It also agreed with [22,44] in Al-Qadisiyah Governorate, where the number of infected female camels was 15 camels with a rate of 88.23%, which is the highest percentage on the percentage of infected males, which amounted to 11.76%, with only two camels out of the studied number [45].

The high incidence of infection in females may be attributed to the fact that the females live longer at slaughter because of the desired benefit in breastfeeding and childbirth, which gives the cyst a greater opportunity for development and growth. Likewise, breeders do not prefer selling or slaughtering productive and young females for the same reason above.

## References

1. Zhang, R.-Q., X.-H. Chen, and H.J.W.j.o.g. Wen, Improved experimental model of hepatic cystic hydatid disease resembling natural infection route with stable growing dynamics and immune reaction. 2017. 23(45): p. 7989.
2. Al-Mayah, K.S., N.M. Al-Bashir ,and B.M.J.M.J.B. Al-Azzawi, In vivo efficacy of *Nigella sativa* aqueous seed extract against metacestode of *Echinococcus granulosus*. 2012. 9(1): p. 140-51.

3. Duman, K., M. Girgin, and S.J.J.G.D.S. Hamcan, Uncomplicated hydatid cysts of the liver: clinical presentation, diagnosis and treatment. 2016. 6(430): p. 2.
4. Athmar, K. and A.J.I.J.A.B.R. Ban-Abbas, Immunization mice with DNA from protoscolices of human hydatid cyst. A immunological study. 2014. 4(1): p. 89-95.
5. Nejad, M.R., et al., Echinococcosis: based on molecular studies in Iran. 2010. 3 (4).
6. Deplazes, P., et al., Global distribution of alveolar and cystic echinococcosis. 2017. 95: p. 315-493.
7. Smyth, J., In vitro culture of *Echinococcus* spp. Proceedings of the 13 th Int. Congr. Hydatidology. Madrid, 1985: p. 84-89.
8. Al-Azawi, A.K., M.A. Fanokh, and R.M. Ali, Comparison of three techniques for DNA extraction from *Echinococcus granulosus* protoscoleces. Int. J. Curr. Microbiol. App. Sci, 2014. 3(11): p. 96-104.
9. Daryani, A., et al., Prevalence of hydatid cyst in slaughtered animals in Northwest Iran. Journal of Animal and Veterinary Advances, 2006.
10. Pakala, T., et al., Hepatic echinococcal cysts: a review. 2016. 4(1): p. 39.
11. Moro, P. and P.M.J.I.J.o.I.d. Schantz, Echinococcosis :a review. 2009. 13(2): p. 125-133.
12. Kadhim, H.A.A. and H.M.H.J.P.A. Al-Mayali, Morphological characterization of *Echinococcus granulosus* isolated from human and sheep in euphrates region of iraq. 2021. 21(1): p. 401-7.
13. Al-Rishawi, K.M. and H.M.J.P.A. Al-Mayali, Molecular detection of *Echinococcus granulosus* strains of human hydatidosis in Al-Muthanaprovince. 2019. 19(2): p. 950-954.
14. Toulah, F.H., et al., Hydatidosis among imported animals in Jeddah, Saudi Arabia. 2017. 4(1): p. 1031.
15. Elmajdoub, L.O. and W.A.J.O.J.o.V.M. Rahman, Prevalence of hydatid cysts in slaughtered animals from different areas of Libya. 2015. 5(01): p. 1.
16. Ebrahimipour, M., et al., Surgically managed human cystic echinococcosis in north-eastern Iran: a single center's experience from 2001 to 2008. 2017. 41(3): p. 883-887.
17. Muqbil, N.A., O.M. Al-salami, and H.A.J.J.J.o.B.S. Arabh, Prevalence of Unilocular Hydatidosis in Slaughtered Animals in Aden Governorate-Yemen. 2012. 5 (2).
18. Jarjees, M. and H.J.I.J.o.V.S. Al-Bakri, Incidence of hydatidosis in slaughtered livestock at Mosul, Iraq. 2012. 26(1): p. 21-25.
19. Mero, W.M., J.M. Jubrael, and A.A.J.S.J.o.U.o.Z. Hama, Prevalence of Hydatid Disease Among Slaughtered Animals in Slemani Province/ Kurdistan-Iraq. 2014 :(1)2 .p. 33-38.
20. Mahmoud, S. and B.J.I.J.o.P. Al-janabi, Incidence of hydatid disease in food animals in Mosul, Iraq. 1981. 5(1): p. 59-60.
21. Al-Ghezi, Z., Epidemiology and diagnosis of hydatid disease in human and ruminant animals in Thi-Qar governorate. 2008, M. Sc. Thesis Thi-Qar University.
22. Rostami, S., et al., Genetic characterization of *Echinococcus granulosus* from a large number of formalin-fixed, paraffin-embedded tissue samples of human isolates in Iran. 2015. 92(3): p. 588.
23. Arif, A., Alameri, A. A., Tariq, U. B., Ansari, S. A., Sakr, H. I., Qasim, M. T., ... & Karampoor, S. (2023). The functions and molecular mechanisms of Tribbles homolog 3 (TRIB3) implicated in the pathophysiology of cancer. International Immunopharmacology, 114, 109581.
24. Lafta, H. A., AbdulHussein, A. H., Al-Shalah, S. A., Alnassar, Y. S., Mohammed, N. M., Akram, S. M., ... & Najafi, M. (2023). Tumor-associated macrophages (TAMs) in cancer resistance; modulation by natural products. Current topics in medicinal chemistry, 23(12), 1104-1122.
25. Qasim, M. T., Fenjan, M. N., & Thijail, H. A. (2022). Molecular identification of *cystoisospora belli* in patients infected with the virus human immunodeficiency. International Journal of Drug Delivery Technology, 12(2), 701-704.
26. Margiana, R., Alsaikhan, F., Al-Awsi, G. R. L., Patra, I., Sivaraman, R., Fadhil, A. A., ... & Hosseini-Fard, S. (2022). Functions and therapeutic interventions of non-coding RNAs associated with TLR signaling pathway in atherosclerosis. Cellular Signalling, 100, 110471.
27. Arif, A., Alameri, A. A., Tariq, U. B., Ansari, S. A., Sakr, H. I., Qasim, M. T., ... & Karampoor, S. (2023). The functions and molecular mechanisms of Tribbles homolog 3 (TRIB3) implicated in the pathophysiology of cancer. International Immunopharmacology, 114, 109581.
28. Lei, Z., Alwan, M., Alamir, H. T. A., Alkaaby, H. H. C., Farhan, S. S., Awadh, S. A., ... & Nekuei, A. (2022). Detection of abemaciclib, an anti-breast cancer agent, using a new electrochemical DNA biosensor. Frontiers in Chemistry, 10, 980162.
29. Bashar, B. S., Kareem, H. A., Hasan, Y. M., Ahmad, N., Alshehri, A. M., Al-Majdi, K., ... & Qasim, M. T. (2022). Application of novel Fe<sub>3</sub>O<sub>4</sub>/Zn-metal organic framework magnetic nanostructures as an antimicrobial agent and magnetic nanocatalyst in the synthesis of heterocyclic compounds. Frontiers in Chemistry, 10, 1014731.
30. Patel, A. A., Asma'a, H. M., Rizaev, J., Mallick, A. K., Qasim, M. T., Al Abdulmonem, W., ... & Ahmad, F. (2024). Application of mesenchymal stem cells derived from the umbilical cord or Wharton's jelly and their extracellular vesicles in the treatment of various diseases. Tissue and Cell, 102415.

31. Qasim, M. T., Mahdi Mohammed Alakkam, E., Mahdi Mohammed, M., Hachim, S. K., Sabah Jabr, H., Emad Izzat, S., ... & D Al-Dhalemi, M. (2022). Ovine Pasteurellosis Vaccine: Assessment of the Protective Antibody Titer and Recognition of the Prevailing Serotypes. *Archives of Razi Institute*, 77(3), 1207-1210.
32. Hjazzi, A., Ahsan, M., Alghamdi, M. I., Kareem, A. K., Al-Saidi, D. N., Qasim, M. T., ... & Mirzaei, R. (2023). Unraveling the Impact of 27-Hydroxycholesterol in Autoimmune Diseases: Exploring Promising Therapeutic Approaches. *Pathology-Research and Practice*, 154737.
33. Hjazzi, A., Nasir, F., Noor, R., Alsalamy, A., Zabibah, R. S., Romero-Parra, R. M., ... & Akram, S. V. (2023). The pathological role of CXC chemokine receptor type 4 (CXCR4) in colorectal cancer (CRC) progression; special focus on molecular mechanisms and possible therapeutics. *Pathology-Research and Practice*, 154616.
34. Gupta, J., Suliman, M., Ali, R., Margiana, R., Hjazzi, A., Alsaab, H. O., ... & Ahmed, M. (2023). Double-edged sword role of miRNA-633 and miRNA-181 in human cancers. *Pathology-Research and Practice*, 154701.
35. Sane, S., Mahoori, A., Abdulabbas, H. S., Alshahrani, S. H., Qasim, M. T., Abosaooda, M., ... & Darvishzadehdadari, S. (2023). Investigating the effect of pregabalin on postoperative pain in non-emergency craniotomy. *Clinical Neurology and Neurosurgery*, 226, 107599.
36. Al-dolaimy, F., Kzar, M.H., Hussein, S.A. et al. (2023). Incorporating of Cobalt into UiO-67 Metal-Organic Framework for Catalysis CO2 Transformations: An Efficient Bi-functional Approach for CO2 Insertion and Photocatalytic Reduction. *J Inorg Organomet Polym*. <https://doi.org/10.1007/s10904-023-02860-0>
37. Muzammil, K., Kzar, M. H., Mohammed, F., Mohammed, Z. I., Hamood, S. A., Hussein, T. K., ... & Alsalamy, A. (2023). Methanol extract of Iraqi Kurdistan Region *Daphne mucronata* as a potent source of antioxidant, antimicrobial, and anticancer agents for the synthesis of novel and bioactive polyvinylpyrrolidone nanofibers. *Frontiers in Chemistry*, 11, 1287870.
38. Ramaiah, P., Baljon, K. J., Hjazzi, A., Qasim, M. T., Salih Al-ani, O. A., Imad, S., ... & Garousi, N. (2024). Dietary polyphenols and the risk of metabolic syndrome: a systematic review and meta-analysis. *BMC Endocrine Disorders*, 24(1), 26.
39. Hsu, C. Y., Ahmed, A. T., Bansal, P., Hjazzi, A., Al-Hetty, H. R. A. K., Qasim, M. T., ... & Elawady, A. (2024). MicroRNA-enriched exosome as dazzling dancer between cancer and immune cells. *Journal of Physiology and Biochemistry*, 1-19.
40. Farhan, S. H., Jasim, S. A., Bansal, P., Kaur, H., Abed Jawad, M., Qasim, M. T., ... & Hadi, A. (2024). Exosomal Non-coding RNA Derived from Mesenchymal Stem Cells (MSCs) in Autoimmune Diseases Progression and Therapy; an Updated Review. *Cell Biochemistry and Biophysics*, 1-18.
41. Kumar, P., Singh, S., Gacem, A., Yadav, K. K., Bhutto, J. K., Alreshidi, M. A., ... & Alam, M. W. (2024). A review on e-waste contamination, toxicity, and sustainable clean-up approaches for its management. *Toxicology*, 153904.
42. Ul Hassan Shah, Z., Bashir, S., Sarfraz, R. M., Mahmood, A., Rehman, U., Ijaz, H., ... & Benguerba, Y. (2024). Development of antihyperlipidemic drug loaded  $\beta$ -CD-based microparticulate carrier systems: tuning and optimization. *Polymer-Plastics Technology and Materials*, 63(11), 1438-1463.
43. Hsu, C. Y., Jasim, S. A., Pallathadka, H., Kumar, A., Konnova, K., Qasim, M. T., ... & Abosaooda, M. K. (2024). A comprehensive insight into the contribution of epigenetics in male infertility; focusing on immunological modifications. *Journal of Reproductive Immunology*, 104274.
44. Zamanian, M. Y., Shahbazi, T., AL-Ghamdi, H. S., Hussien, B. M., Alghamdi, M. A., Qasim, M. T., ... & Golmohammadi, M. (2024). Chemopreventive and Anticancer Role of Resveratrol against non-melanoma skin cancer (NMSC): Focusing on cellular and molecular mechanisms and biochemistry. *Authorea Preprints*.
45. Hjazzi, A., Jasim, S. A., Al-Dhalimy, A. M. B., Bansal, P., Kaur, H., Qasim, M. T., ... & Zwamel, A. H. (2024). HOXA9 versus HOXB9; particular focus on their controversial role in tumor pathogenesis. *Journal of Applied Genetics*, 1-20.