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Molecular diagnosis of Toxoplasma gondii in aborted women and lambs using PCR technology

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Abstract: The current study included the measurement of genes B1, the GRA6 gene, and the SAGA2 gene in sheep and women infected with toxoplasmosis. Forty-five samples were collected from the female sheep and 15 samples from the women. Molecular variables were measured by PCR technique. The results showed the appearance of the B1 gene in women by 73% and in sheep by 71%. As for the GRA6 gene, the incidence of the gene was 53% in women and 66% in sheep, while the SAGA2 gene was 0% in women and sheep.

Keywords: Toxoplasmosis, B1 gene, GRA6, SAGA2.

Introduction: *Toxoplasma gondii* is the most common zoonotic disease born in meat affecting all warm-blooded animals and humans. Sheep are one of the main reservoirs of *T. gondii* worldwide, and infection leads to various cascades, such as abortion (Deljavan *et al*.,2022). *T. gondii* is transmitted to humans through ingestion of undercooked meat containing tissue cysts. *T. gondii*. Foods or drinks contaminated with oocysts are also a source of contamination but cats are the only definitive hosts for this parasite in which sexual reproduction occurs and are able to secrete an environmentally resistant oocyst-stage, while all mammals, including humans and birds, serve as intermediate hosts (Kolören and Dubey., 2020). *T. gondii* is a major cause of abortion-related reproductive failure, especially in sheep and goats, which are the main source of red meat in many countries worldwide due to its nutritional benefits with adequate amount of dietary protein (Al-Alo and mohammed.,2021). Toxoplasmosis has been associated with public health and economic concerns due to miscarriage in humans and animals (Elaadli *et al*., 2023).

Materials and Methods: Blood samples to be studied for women were collected from Tikrit Teaching Hospital and medical laboratories for the period from October 2021 to March 2022. The study included 15 samples from women, and blood samples were collected in test tubes consisting of 3 ml of blood samples designated for examination. Molecular, where the samples were placed in test tubes containing the anticoagulant EDTA tube, and the patient's name was written on each tube. As for sheep, blood samples were collected for aborted female sheep from veterinary clinics in Tikrit, in addition to field visits accompanied by veterinarians to villages and rural areas to collect samples within the same time period. For aborted lambs, each tube was numbered with a special number and samples were left at room temperature and then placed in a refrigerated container and transported to the laboratory. The serum was separated by using a centrifuge at a speed of 3000 revolutions per minute for 10 minutes, then the serum was withdrawn with a Pasteur pipette and the serum was placed in small, sterile plastic tubes, Eppendorf tube, and then the samples were kept in the freezer at -20ºC until the necessary tests were performed. Molecular examinations were carried out in the central laboratories of the University of Tikrit.

1_ DNA Extraction: DNA was isolated from blood samples of each woman infected with toxoplasmosis, as well as sheep infected with toxoplasmosis, for the purpose of DNA extraction using the preto minig DNA kit equipped by Geneaid company.

Genotyping:

1- Reducing the initials

1- The primers were designed from the Korean manufacturer (Macrogen) in the form of a powder with a concentration of 250 pcomol / microliter. The primers were dissolved by adding 250 microliters of nuclease free water, according to the instructions of the processing company.

2- Dilute the primer and obtain a concentration of 10 picomoles/microliter by adding 10 microliters of the buffer solution to 90 microliters of nucleic acid-free water.

2 PCR polymerase reaction:

The polymerase chain reaction was carried out using a thermocycler equipped by (Applied biosystem) a Singaporean company.

Primers for the PCR reaction: The PCR technique was used to detect the presence of the Toxoplasma gondii parasite DNA by using specialized primers designed for this purpose as follows:

Results and Discussions:

B1 gene in female and female lamb:

Confirmation of infection with *T. gondii* for women was done by a polymerase chain reaction (PCR) test using a thermal cycler, as a piece of the gene called B1 of size 182bp was used in this test, and this piece was duplicated using specific primers. The results showed the presence of this gene in the samples that were isolated from women who were confirmed to be infected with toxoplasmosis. The primers of the B1 gene were used in molecular tests of the DNA that was isolated from the blood samples that were collected to detect the presence of the parasite and diagnose the infection. The results showed the presence of a genetic piece with a length of 182 bp in the positive samples. The B1 gene was used in many previous studies in diagnosing *T. gondii* infection, after studies proved that they have a specificity and high sensitivity in diagnosing the *T. gondii* parasite, in addition to that the B1 gene is known as the *T. gondii* strain found in Iraq. . These results are consistent with the results that appeared in the studies conducted by (Hadraawy & Hadi, 2017 ;Chabbert *et al*., 2004).

The results of the current study are higher than the results of the researcher Asgari and his group (2013) in Iran 14.4%, and Shaker and his group (2018) in Diyala 15%. The difference in the results between the current study and other studies is due to the fact that the study that gave similar results used the conventional PCR (conventional PCR), but the contrasting studies used the RT PCR test, which recorded a sensitivity and specificity in diagnosis estimated at 100% (Verweij *et al*., 2004).).

The reason may be attributed to a difference in sampling, the number of samples under study, the geographical location and the surrounding environmental conditions. The reason may be due to the recent spread of the phenomenon of keeping pets in homes, especially cats. As for the female sheep, the results of the PCR test showed an infection rate with *T. gondii* amounted to 73% (32 samples) positive from the total samples of the total blood.

The results of the current study are in agreement with the results of the researcher, as the incidence of the B1 gene in samples taken from the heart of sheep was 78% in Isfahan, Iran, and higher than what was reached by the researcher Issad and his group (2020) in Algeria, where the incidence of the B1 gene in sheep was 35.24%, as well as contrary to what was found The researcher Deljavan and his group (2022), as the infection rate reached 11.11% for sheep milk samples, may be due to the fact that the researchers took samples of male and female sheep, as well as our molecular study of blood samples, which is more accurate in diagnosis, and also because of the difference in the total number of samples examined.

The GRA6 gene in female and female lamb:

The results of the PCR test showed that the rate of infection with *T. gondii* was 53% (8 samples) positive of the total blood samples. The result was similar to that of the researcher Al-Hadraawy *et al.*in the year (2019), as the percentage of the appearance of the gene in women reached 60%, and contrary to what was reached by the researcher Singh and his group (2022), as the percentage of the appearance of the gene in women reached 16.7% (12/72) samples. A reason may be attributed to the fact that women are in direct and continuous contact with sources of infection with *T. gondii* while cutting meat while cooking and washing vegetables and fruits that are likely to contain infectious stages of the parasite.

The result was similar to what the researcher Nosrati reached in the year (2020), as the researcher showed that 30 samples out of 44 brain samples were infected, as the gene expression rate reached 66.18%. Sheep 16.6%. The study of the GRA6 gene in sheep is one of the few recent studies and researches. The reason for the difference can be attributed to the nature of the food you eat and the environment in which you are present, as well as to the different laboratory conditions, examination methods, sample collection method, sample preservation, and the speed of conducting the analysis.

SAGA2 gene in female and female lamb:

The results of the current study showed that the gene did not appear in the samples examined for women, as well as in the female sheep.

Table (1): B1, GRA6, and SAG2 gene primers

Primer	Sequence	Size
B1 gene	5'ATAGGTTGCAGTCACTGACG3' 5' CTCCTCTTC GCGAAACCTCA3'	182
GRA6	3-ATTTGTGTTTCCGAGCAGGT5 5-GCACCTTGGCTTGTGGTT3	246
SAG2	5- GCACTGTTGTCCAGGGTTTT3 5- GGAACGCGAACAATGAGTTT3	344

The samples were placed in a thermocycler to conduct a PCR reaction according to the program designated for the reaction for each primer as shown in Table (2).

Table (2): The program used in the polymerase chain reaction (PCR) for all primers.

stage	type of gene	number of cycles	time	temperature
Initial deformation	B1 gene	1	5sec	94cº
metamorphosis		35cycle	30sec	94 cº
Initial link			30sec	59 cº
Elongation			30sec	72 cº
Final elongation		1	7min	72 cº
Initial deformation	GRA6 SAGA2	1	4min	95cº
metamorphosis		40cycle	30min	95cº
Initial link			1min	56cº
Elongation			2min	72cº
Final elongation		1	7 min	72cº

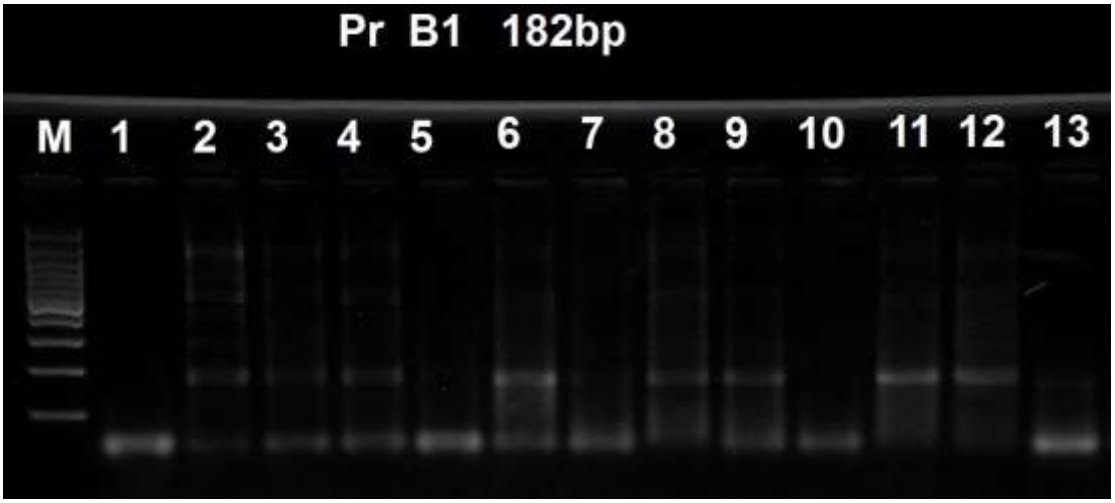

Figure 1: Duplications of the B1 gene segment using polymerase chain reaction from DNA extracted from women. Routes 1 to 13 show the results of double B1 gene from samples extracted from women, so that the cutoff size was 182 bp. The M pathway represents the 100bp ladder of DNA.

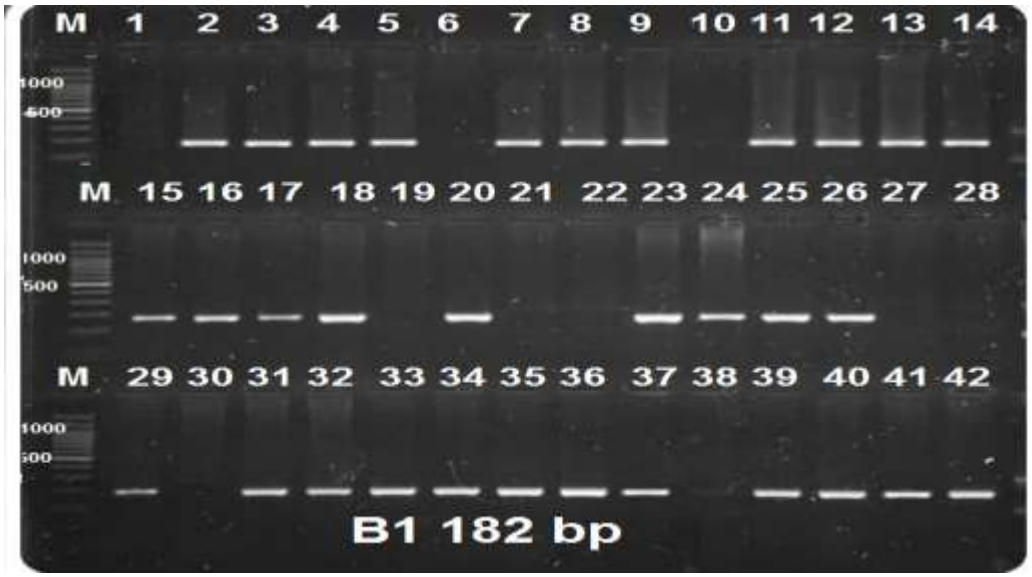

Figure 2: Duplication products of the B1 gene segment using polymerase chain reaction from DNA extracted from a female lamb. Tracks 1 to 42 show the results of doubling the B1 gene from samples extracted from sheep, so that the cutoff size was 182bp. The M track represents the 100 bp ladder of the DNA molecular size, and it was 71%.

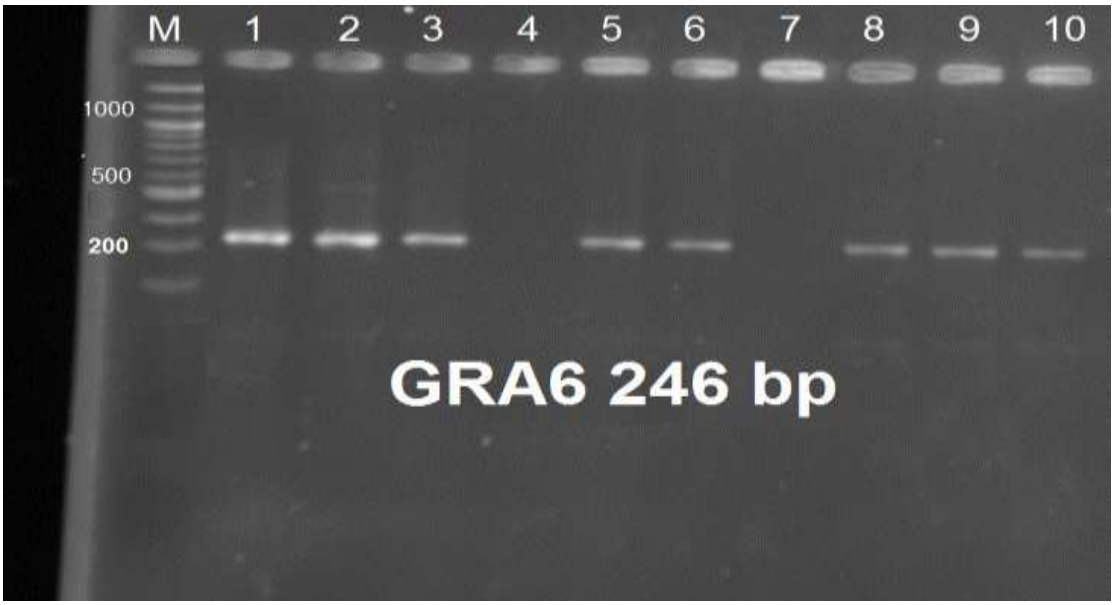

Figure 3: Duplication products of the GRA6 gene segment using polymerase chain reaction from DNA extracted from women. Routes 1 to 13 show the results of GRA6 gene duplication from samples extracted from women, with a cutoff size of 246 bp. The M pathway represents the 100 bp ladder of DNA.

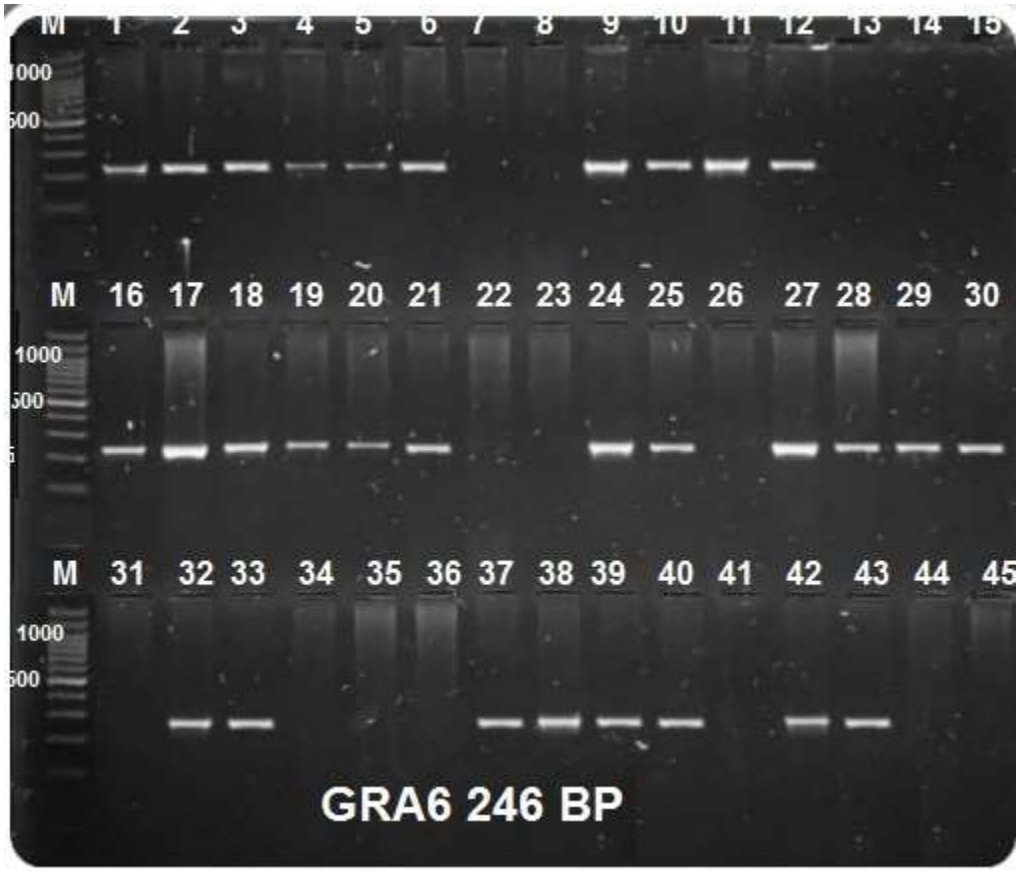

Figure 4: Duplication products of the GRA6 gene segment using PCR from DNA extracted from lamb. Routes 1 to 45 show the results of double GRA6 gene from samples extracted from a female lamb, where the size of the pieces was 246 bp. The M pathway represents the 100 bp ladder of DNA.

References:

- Al-Hadraawy, S. K., Alzeyadi, M., Shakir, A. A., & noori Al-dujaili, A. (2019, July). Detection of Toxoplasma gondii genotypes in abortion women by RFLP-PCR in Al-Najaf Al-Ashraf province. In *Journal of Physics: Conference Series* (Vol. 1234, No. 1, p. 012084). IOP Publishing.

- Kareshk, T. A., Mahmoudvand, H., Keyhani, A., Oliaee, T. R., Mohammadi, M. A., Babaei, Z., ... & Zia-Ali, N. (2017). Molecular detection and genetic diversity of Toxoplasma gondii in different tissues of sheep and goat in Eastern Iran. *Tropical Biomedicine*, *34*, 681-690.

- Singh, B., Debrah, A. Y., Debrah, L. B., & Djameh, G. (2022). Prenatal Diagnosis and Genetic Characterization of Toxoplasmosis in Immunocompetent and Immunocompromised Pregnant Women and the Risk of Congenital Toxoplasmosis: A Prospective Study in Ghana. *Journal of Science and Technology (Ghana)*, *41*(2), 18-42.

_ Deljavan, N., Moosavy, M. H., & Hajipour, N. (2022). Molecular detection of toxoplasma gondii DNA in goats (Capra hircus), sheep (Ovis aries), and donkey (Equus asinus) milk using PCR in East Azerbaijan province, Iran. *Research in Veterinary Science*, *152*, 58-60.

_ Kolören, Z., & Dubey, J. P. (2020). A review of toxoplasmosis in humans and animals in Turkey. *Parasitology*, *147*(1), 12-28.

_Al-Alo, KZ, & Mohammed, AJ (2021). A cross sectional study on the seroprevalence of bovine brucellosis in Al-Najaf province in Iraq. *Iraqi Journal of Veterinary Sciences* , *35* (4), 617-620.

_Elaadli, H., Abo El-Makarem, H., Abd Elrahma, A. H., Shaapan, R. M., & Bessat, M. (2023). Prevalence and associated risk factors of Toxoplasma gondii infection in sheep and aborted women in Egypt. *Iraqi Journal of Veterinary Sciences*, *37*(2), 437-445.

-Al-Hadraawy, S. K. and Hadi F. A. (2017). Immunological and molecular sudy of *Toxoplasma gondii* in Al-Najaf Governorate-Iraq. International Journal of Pharmacognosy and Phytochemical Research 9(4): 482-492.

-**Asgari, Q., Fekri, M., Monabati, A., Kalantary, M., Mohammadpour, I., Motazedian, M. H., & Sarkari, B. (2013).** Molecular genotyping of *Toxoplasma gondii* in human

spontaneous aborted fetuses in Shiraz, Southern Iran. *Iranian journal of public health*, *42*(6), 620.

-**Chabbert, E.; Lachaud, L.; Crobu, L.; Bastien, P. (2004).** Comparison of two widely used PCR primer systems for detection of *Toxoplasma* in amniotic fluid, blood, and tissues. J. Clin Microbiol 2004; 42: 1719-22.

Govindarajan, S., Mustafa, M. A., Kiyosov, S., Duong, N. D., Raju, M. N., & Gola, K. K. (2023). An optimization based feature extraction and machine learning techniques for named entity identification. Optik, 272, 170348.

Hassan, J. A., & Rasheed, M. K. (2022, November). Synthesis and characterization of some benzimidazole derivatives from 4-methyl ortho-phenylene diamine and evaluating their effectiveness against bacteria and fungi. In AIP Conference Proceedings (Vol. 2394, No. 1). AIP Publishing.

-Issad, N. A., Abdelouahed, K., Bekhouche, S., Boubeuker, R., Adjmi, H. H., Ouchene-Khelifi, N. A., ... & Khelef, D. (2020). Molecular detection of the B1 gene of Toxoplasma gondii in blood samples of female sheep and goats in Tebessa, northeastern Algeria. *Comparative Immunology, Microbiology and Infectious Diseases*, *72*, 101530.

Karupusamy, S., Mustafa, M. A., Jos, B. M., Dahiya, P., Bhardwaj, R., Kanani, P., & Kumar, A. (2023). Torque control-based induction motor speed control using Anticipating Power Impulse Technique. The International Journal of Advanced Manufacturing Technology, 1-9.

-Nosrati, M. R. C., Shemshadi, B., Shayan, P., Ranjbar, S., & Bahadori, A. E. (2020). High prevalence of Toxoplasma gondii infection in ovine aborted fetuses in Gilan Province, Iran: Molecular detection and genotype characterization. *J. Basic Res. Med. Sci.*, *7*, 53-62.

-Shaker, M. J., Darweesh, N. H., Hussein, R. A., & Salman, S. T. (2018). Immunological and Molecular study of *Toxoplasma gondii* from aborted women in Diyala/Iraq.

Sudha, I., Mustafa, M. A., Suguna, R., Karupusamy, S., Ammisetty, V., Shavkatovich, S. N., ... & Kanani, P. (2023). Pulse jamming attack detection using swarm intelligence in wireless sensor networks. Optik, 272, 170251.

-**Verweij, J.J. ; Blange, R.A. ; Templeton, K. ; Schinkel, J. ; Brienen, E.A.T ; van Rooyen, M.A.A. ; van Leishout, L. ; Polerman, A.M. (2004).** Simulatnous detection of *Entamoeba histolytica, Giardia lamblia,* and *Cryptosporidium parvum* in fecal samples using multiplex real-time PCR. J. Clin. Microbiol., 42(3): 1220–1223.

Nijris, O. N., Khaleel, Z. I., Hamady, S. Y., & Mustafa, M. A. (2020). The effectiveness of Aqueous Extract of Grape Seeds Vitis vinifera as an antibiotic for some microorganisms and

its Protective Role Histology for Liver, Kidney in Mice. Indian Journal of Forensic Medicine & Toxicology, 14(2), 1838-1845.

Mustafa, H. A., Majid, H. H., Abdulqader, A. T., Mustafa, M. A., & Salih, A. A. (2019). Study On Some Physiological, Biochemical And Hormonal Parameters Of Seminal Fluid Of Infertile Men. Biochem. Cell. Arch, 19(Supplement 1), 1943-1947.

Fadhil, K. B., Majeed, M. A. A., & Mustafa, M. A. (2019). Electronic study of fresh enzyme complexes of antifungal drugs-P450 and Aspergillus kojic acid biosynthesis. W: w saccharose flavus: fructose as a substratum. Annals of Tropical Medicine and Health, 22, 65-72.

Abdulazeez, M., Hussein, A. A., Hamdi, A. Q., & Mustafa, M. A. (2020). Estimate the Complications That Resulting from Delayed Management of Dental Trauma in Tikrit City. Journal of Cardiovascular Disease Research, 11(2), 80-82.

Hama Hasan, T. A., Erzaiq, Z. S., Khalaf, T. M., & Mustafa, M. A. (2020). Effect of Equisetum Arvense Phenolic Extract in Treatment of Entamoeba Histolytica Infection. Systematic Reviews in Pharmacy, 11(11).

Hama Hasan, T. A., Erzaiq, Z. S., Khalaf, T. M., & Mustafa, M. A. (2020). Effect of Equisetum Arvense Phenolic Extract in Treatment of Entamoeba Histolytica Infection. Systematic Reviews in Pharmacy, 11(11).

Nijris, O. N., Khaleel, Z. I., Hamady, S. Y., & Mustafa, M. A. (2020). The effectiveness of Aqueous Extract of Grape Seeds Vitis vinifera as an antibiotic for some microorganisms and its Protective Role Histology for Liver, Kidney in Mice. Indian Journal of Forensic Medicine & Toxicology, 14(2), 1838-1845.

Ali, A., Jassim, A.F., Muhsin, S.N., & Mustafa, M.A. (2020). Study of Lycium Shawii Phenolic Compounds in Treatment of Hyperlipidemia. Journal of cardiovascular disease research, 11, 196-199.

Ibrahim, H. M., Jumaah, L. F., Khalaf, S. A., & Mustafa, M. A. (2021). KNOWLEDGE AND PRACTICE OF BREASTFEEDING AND WEANING IN MOTHERS LIVES SAMARRA CITY, IRAQ. Biochemical & Cellular Archives, 21.

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