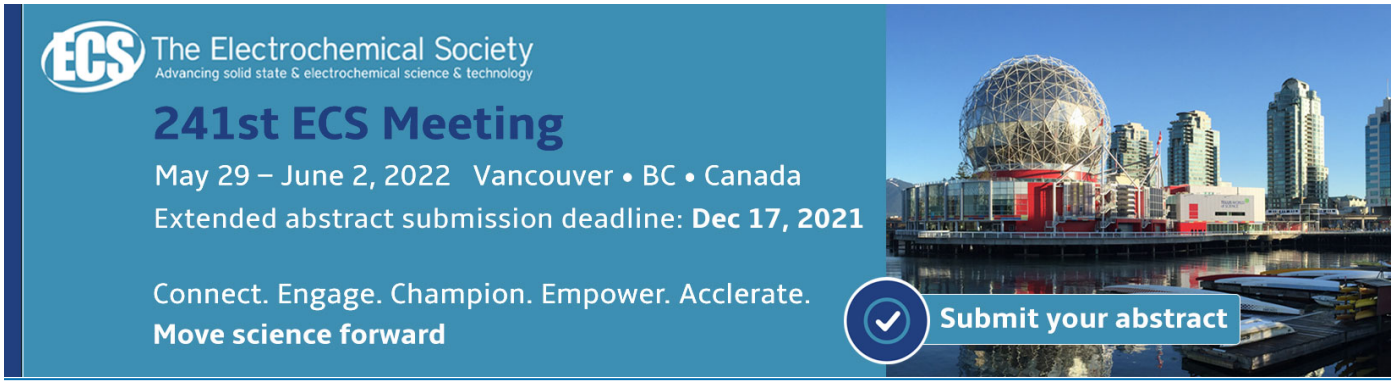
An Epidemiological and Therapeutic Study of *Fasciola hepatica* Parasite in Goats of Anbar Province-Iraq



An Epidemiological and Therapeutic Study of *Fasciola hepatica* Parasite in Goats of Anbar Province- Iraq

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Abstract. This study was conducted to find out the prevalence of *Fasciola hepatica* in goats in Anbar province-Iraq, via routine examination of the livers taken from goats slaughtered in central slaughterhouses in the province to ensure that they were infected with hepatic helminths. The results indicated that a total infection rate of 43.5%. Ramadi area had the highest infection rate, moreover, the highest infection rate was 69.9% for ages 6-8 years. Significant differences were found in the average numbers of helminths in the bile sac and liver of both males and females of infected goats. On the other hand, It has been proven that the concentrations of (0.5-10) mg / ml of the alcoholic extract of the plant *Aizoon hispanicum* had a significant effect on the vitality of the helminths in vitro, as the concentration 10 mg/ml leads to the complete inhibitory for the vitality of helminths where after 36 hours it led to a 100% killing rate. The current study concluded that the goats in the study areas are infected with the *F. hepatica* parasite at a high rate, and this calls for finding safe treatment methods using medicinal herbs and plants because they contain many active compounds that affect the helminths and lead to their death, and they are safe strategic alternatives to harmful chemical drugs. This necessitates the establishment of an integrated control approach through treatment with effective plant extracts to eliminate these parasites and their health and economic damage.

1. Introduction

Infection with the *Fasciola hepatica* (liver flukes) is one of the most dangerous parasitic diseases that afflict various kinds of animals and lead to health damage and heavy economic losses represented in the deterioration of the growth of animals and sometimes their death [1]. It causes Fascioliasis, which leads to many disease symptoms and serious disease effects that begin with a lack of meat and milk production [2]. Chronic cases cause cirrhosis of the liver, lack of weight animal, decreased production of wool, anemia, and damage to the affected livers, and death of affected animals [3]. *F. hepatica* parasite needs two hosts to complete its life cycle, the first host is the intermediate host, a species of Mollusca belonging to the genus *Lymnaea* [4]. The second host is the definitive host and includes many species of herbivorous mammals such as goats, cows, camels, antelope, horses, deer, mice, rabbits, and monkeys as well as humans [5].

The infection occurs as a result of eating herbs or water contaminated with the infectious phase, which is the metacercaria larva [6]. which travels inside the digestive system and reaches the stage of developing in the intestine and penetrates the intestinal wall heading to the liver to develop there to the adult worm within several weeks of the animal being infected [7]. Although *F. hepatica* has a veterinary and economic medical importance as a result of infecting many animals, they have become

a major health problem for humans as well, as 2.4 million people have been infected and the risk of infection threatens about 180 million people around the world [8]. *F. hepatica* adults live in the biliary ducts of the liver and the bile sac [9]. Infection is spread in various countries of the world, especially in tropical and subtropical regions, including Iraq, especially in the southern and central regions, where the intermediate host is abundant, where goats and cattle have raised that feed on plants carrying metacercaria [10]. Several studies have been conducted to investigate the *F. hepatica* in goats in many countries around the world. For example, in Pakistan, an infection rate of 32.02% was recorded [11]. In another study, a 68.4% infection rate was recorded in goats of southwestern Tunisia [12]. While it was found that the infection rate is 6.6% among the examined goats in Mazandaran, northern Iran [13]. As for Iraq, a study conducted in the Qadisiyah province, which recorded a rate of 42.5% [14], in addition to, an infection rate of 6.3% was recorded in Duhok province, north Iraq [15]. As well, Al-khafaji et al., [16] mentioned an infection rate of 1.3% in Babil province.

Many wild herbs and medicinal plants were used in the treatment of parasitic diseases due to the effectiveness of their chemical compounds against the pathogens [17]; [18]. The plant *Aizoon hispanicum* belongs to the Aizoaceae family, and its common name is Ghossol plant, which is an annual herb with upward stems ranging from 5-18 cm high, the plant grows on the gravel lands adjacent to the river far from the agricultural fields, as well, it is found in calcareous and sandy soils, and blooms from early March to late May, its chemical contents are saponins, sterols, alkaloids, glycosides, and other important compound is Coumarins, which is used in the perfumery, and because it contains Sterol, which is a solid alcoholic substance, it is used as a disinfectant for wounds, also, it is used to treat parasitic helminths [19]. From the studies conducted to treat Fascioliasis, Alvarez-Mercado et al., [20] used the fifteen tropical botanical extracts on *F. hepatica* in vitro. And in a study done by Ezeta-Miranda et al., [21] to evaluate the efficacy of purified fractions of *Artemisia ludoviciana Nutt. mexicana* and ultrastructural damage to newly excysted juveniles of *F. hepatica* in vitro. On the other hand, Ghafar et al., [22] also studied the anti-helminthic activity of *Zingiber officinale roscoe* extract on *F. hepatica* miracidia in vitro.

Fascioliasis disease causes great economic losses and leads to a decrease in animal production and the death of large numbers of animals, so this study was conducted to determine the extent of the prevalence of *F. hepatica* in goats in Anbar province and to find a safe and effective treatment for this disease using medicinal plants and herbs, the alcoholic extract of the *Aizoon hispanicum* plant was used because it contains many effective compounds, which are considered a good strategic alternative to chemical drugs that harm human health, animals. This study provides a basis for exploring the therapeutic potential of these medicinal plants against parasitic diseases. However, extensive studies are needed to determine the effectiveness of the active compounds of other plants, which may lead to a significant addition of value in the pharmaceutical industry, and to provide a cost-effective method of treatment with minimal side effects.

2. Materials and Methods

2.1. Examination of goat livers

A total of 455 liver of goats (231 males, 224 females) were examined depending on what was slaughtered in the meat sale slaughterhouses in some areas of Anbar province, which included (Ramadi, Khaldiya, Qaim, and Al-Baghdadi) with ages ranging from 3 months - 9 years, where the goat livers were collected and laid In plastic containers and brought to the Parasitology Laboratory in the Department of biology -College of Science for the purpose of its examination, a blade was used to dissect the livers from the bile duct region to detect and diagnose *F. hepatica* parasite according to Kendal method [23].

2.2. Preparation of the alcoholic extract of the Ghossol plant

The Ghossol plant (Figure 1) was collected from Al-Baghdadi area, west of Ramadi city, which was diagnosed in Anbar University Herbarium. After cleaning and drying the plant parts, the alcoholic extract was prepared according to He et al., method [24] with a weight of 25 g of the dried plant parts and it was dissolved in 250 ml of ethyl alcohol at a concentration of 70% at a ratio 1: 10. Then, the mixture was placed in the Magnetic stirrer for homogeneous mixing and left for 24 hours at room temperature. After that, the solution was filtered in Buchner Funnel, the filtrate was collected and evaporated using a Vacuum Rotary evaporator at a temperature of 60 ° C to get rid of the solvent.

The solution was filtered by filter paper to get rid of the chlorophyll dye and the extract was returned to an evaporator to get rid of water, then the mixture was sterilized using the pasteurization process at 62 °C for 10 minutes, with this, the standard solution (stock) of the alcoholic extract of the plant was obtained from which the concentrations 0.5, 2.5, 5, 10 mg/ml used in this study were prepared.

Figure 1.The Ghossol plant *Aizoon hispanicum*

2.3. Chemical detection of active compounds in plant [25]

Detection of tannins: A few drops of lead acetate solution1% were added to 5 ml of the plant extract, the appearance of white gelatin deposits indicates the presence of tannins.

Detection of phenols: 1 ml of dry plant extract was added to 1 ml of a 1% ferric chloride solution, the appearance of green or blue color indicating the presence of phenols.

Detection of the saponins: 3 ml of mercuric chloride solution 1% was added to 5 ml of the plant extract, the appearance of the white deposit indicated the presence of saponins.

Detection of Flavonoids: A 10 ml solution of 50% ethanol was added to 10 ml of 50% potassium hydroxide and mixed in an equal amount, the presence of a yellow color indicated the existence of Flavonoids.

Detection of Glycosides: 1 g of a dry plant extract was mixed with 10 ml distilled water, added a few drops of Fehlink reagent, the appearance of red indicates the presence of Glycosides.

Detection of Fuocumarins: two equal amounts of a plant extract were mixed with 1% potassium hydroxide, the appearance of greenish-yellow indicates the presence of Fuocumarins.

Detection of Terpenes: A mixture of the following chemical materials was prepared: one gram of plant extract was dissolved in 2 ml of chloroform, followed by a drop of anhydrous acetic acid and a drop of concentrated sulfuric acid, the presence of brown sediment indicated the existence of Terpenes.

Detection of Alkaloids: 10 grams of plant extract was boiled with 50 ml of distilled water containing 4% hydrochloric acid *(*HCL). The solution was cooled and filtered. Following this, 0.5 ml of leachate was tested in a watch glass with 0.5 ml of Meyer reagent, the appearance of white deposits indicates the presence of Alkaloids.

Detection of volatile oils: A few drops of plant extract were added to the filter paper to reduce saturation and exposure to ultraviolet radiation, the appearance of a grayish color indicates the presence of volatile oils.

2.4. Effect of plant alcoholic extract concentrations on viability and size of F. hepatica helminths

After preparing the required concentrations of the alcohol extract 0.5, 2.5, 5, and 10 mg/ml, 20 helminths were placed in each Petri dish containing 20 ml of the alcoholic extract dissolved in sterile Normal saline, and with three replications for each concentration, in addition to the control treatment containing 20 ml of sterile Normal saline only. Petri dishes were placed in an incubator at 37 ° C and were followed up at different intervals of 12, 24, 36, and 48 hours according to Jeyathilakan et al., method [26] by measuring their length and width using a graduated ruler.

Also, the vitality of the helminths before and after the treatment was compared with different concentrations of the plant extract by observing the dead helminths, which were characterized by immobility, inactivity, shrinkage, and their small size, while the full vitality of the helminths appeared in the control treatment (Figure 2).

2.5. Statistical analysis

The data collected on disease prevalence were analyzed statistically using different statistical techniques by Statically Analysis Software (SAS) application (proc) for Windows version 10 [26]. The prevalence of mange was assessed using descriptive statistics.

Chi-square was applied to confirm disease spread based on sex, regions, and age; the results were compared using the least significant difference (L.S.D.). For the therapeutic study, results and data generated from the trial were expressed as mean ± standard error for continuing variants, using the one-way analysis of variance (ANOVA) table, at a probability level $P<0.05$.

3. Results and Discussion

Fascioliasis in the liver of domestic ruminants is a group of important parasites in the world due to the great economic losses it causes that result from the destruction of the infected liver. This study is the first of its kind in a country where the Ghossol plant is used to treat *F. hepatica* parasites. The results showed that goats in Anbar province were infected with F. hepatica parasites by 43.5%, where Ramadi region recorded the highest rate of 59.5%, while Khaldiya region recorded the lowest rate of 20.8%, with significant differences (Table 1). which is close to the rate found by Abdul Qadir [14], which reached 42.5% in Al-Qadisiyah province. However, it was higher than Mikaeel [15] which was 6.3% in Duhok province and also higher than that recorded by Zafar et al., [11] which was 32.02% in Pakistan. But it is lower than that recorded by Hamami et al., [12] in southwestern Tunisia, which amounted to 68.4%.

The difference in the infection rate between this study and the previous studies is due to the different areas studied, the different grazing, breeding and feeding habits of the animals examined in the livestock fields, and the difference in following the rules of hygiene and health conditions for each of the animals raised in the breeding pens [28]. The reason for the variation in infection rates in the studied areas is due to the difference in the environment between those areas. As the city of Ramadi is close to lakes and rivers, where there are many intermediate hosts for these parasitic worms,which contributes to the high rate of infection, as well as the difference in the method of raising animals, including goats, due to the animals that are grazed in agricultural fields with a higher rate of infection than those raised in pens [29]. In addition, studies conducted in different countries revealed that the prevalence of fascia varies from one region to another, and is influenced by environmental conditions, animal species, and breeding method [30].

It was observed that the infection rate was higher among female goats, reaching 51.7%, compared to the infection rate for males at 35.4%, with significant differences ($p\leq0.05$) (Table 2). This is consistent with the study of Shahatha [31], the reason for this is because most of the animals that are slaughtered are males at young ages, and this reduces the chances of them being exposed to parasitic

infections, as livestock keepers keep females for longer periods for purposes of reproduction and increase the offspring and this raises the infection rate in addition to the males feeding on forage Equipped with regular protein therapy against helminths and other pathogens [32].

Moreover, it was found that the incidence of the infection increases with age, and the statistical analysis showed a significant difference ($P < 0.05$) with regard to the effect of age on infection, as the highest incidence of hepatic worms infection, was 69.9% for ages 6-8, while the lowest was 22.2% for ages from One to two years (Table 3). this is in agreement with the results of Hassan et al., [33] during his study of black Bengal goats in Chittagong, Bangladesh, it also, corresponds to a study by Pérez-Creo [33] on goats from northwest Spain, this is due to the occurrence of infection with *F. hepatic* parasites in goats at young ages and does not begin to appear until the animal is old.

A significant difference ($p \leq 0.05$) was observed between the average numbers of helminths in the liver and bile sac, as it reached (15-450) and (15-260) helminths in the liver for both females and males, respectively, while the average number of helminths in the bile sac ranged from (5- 65) and (5-45) helminths in both females and males, respectively (Table 4). This indicates that the severity of infection with *F. hepatic* parasite is higher in female goats than in males, this is consistent with the findings of Shahatha [31] in Anbar province, this is attributed to the fact that the average life span of females is higher than that of males, as well as that most of the livers examined had chronic conditions that lead to the accumulation of large numbers of helminths in both the liver and the bile sac.

The results showed the effect of different concentrations of alcoholic extract of A. hispanicum plant and its effectiveness in inhibiting the viability of *F. hepatica* helminths (Table 5), where the concentration of 10 mg/ml showed the highest inhibitory effect on the helminths' vitality, where after 36 hours it led to a 100% killing rate, meaning that the viability rate was 0%. As for the lowest concentration of 0.5 mg/ml, it led to the least effect on helminths' vitality, reaching 100, 100, 88.2, and 2.1% during 12, 24, 36, and 48 hours, respectively. While the rate of helminths' vitality was 98.5, 90.1, 72.8, 0% at a concentration of 2.5 mg/ml, whereas the inhibitory effect of the helminths' vitality increased when using the concentration of 5 mg/ml, as the vitality rate reached 60.4, 35.5, 11, and 0% during the above consecutive exposure periods.

The viability of parasitic worms before and after treatment was compared with different concentrations of plant extracts by observing the dead helminths, which were distinguished by their rigidity, inactivity, shrinkage and small size, while the full vitality of helminths appeared. In the control treatment (Figure 2) , this is in agreement with the study of Alvarez-Mercado [20]. The inhibitory activity of the alcoholic extract of *A. hispanicum* plant on the helminths' vitality in vitro, is due to, it contains many active compounds, saponins, sterols, alkaloids, glycosides, Coumarins, and tannins, which penetrate the living tissues of the helminths and affect the Enzyme-making sites and lead to their stop. It also disrupts the work of the enzyme acetylcholinesterase, which controls the vital activities of the parasite, and this causes the helminths' death [35]. The parasitic helminths *F. hepatica* looked like leaves, greenish-brown in color, 30 mm in length, 13 mm in width, and had a conical protrusion from the front side and broad shoulders (Figure 3).

Table 1. Percentage of infection with F. hepatica according to regions.

Region	Number of examined goats	Number of goats affected	%
Ramadi	131	78	59.5
Khalidiya	116	57	49.1
Qaim	120	25	20.8
Al-Baghdadi	88	38	43.1
Total	455	198	43.5

Table 2. Percentage of infection with F. hepatica according to Sex.

Sex	Number of goats	Number of goats	%

	examined	affected	
Males	231	82	35.4
Females	224	116	51.7
Total	455	198	43.5

Table 3. Percentage of infection with F. hepatica according to age.

Age (year)	Number of goats examined	Number of goats affected	%
1 Month - 2	99	22	22.2
3-5	120	27	22.5
6-8	113	79	69.9
9-11	123	70	56.9
Total	455	198	43.5

Table 4. Average of numbers of F. hepatica helminths in liver and bile sac for both males and females of infected goats.

Period of exposure (hours) Concentration mg / mL	*** Average ± standard error**			
	12	24	36	48
control	100±0.0 a	100±0.0 a	99.2± 0.0 a	95.0±0.1 a
0.5	100±0.0 a	100±0.0 a	88.2± 1.1 a	2.1± 0.3 b
2.5	98.5±1.0 a	90.1±0.2 a	72.8± 0.5 b	0.0 c
5	60.4±1.2 b	35.5±0.5 b	11±2.0 c	0.0 c
10	7.1±0.2 c	1.5±0.5 c	0.0 d	0.0 c

Table 5. Effect of alcoholic extract concentrations of A. hispanicum plant on the viability of F. hepatica, in vitro.

Infection area	Average of helminths' numbers	
	Males	Females
liver	15 - 260	15 – 250
bile sac	5 - 45	5 – 65

Notes: The number represents the rate of 3 replicates ± the standard error
Notes: Different characters within the column indicate significant differences between the coefficients within the level of significance ($p \leq 0.05$) according to the test (L.S.D)

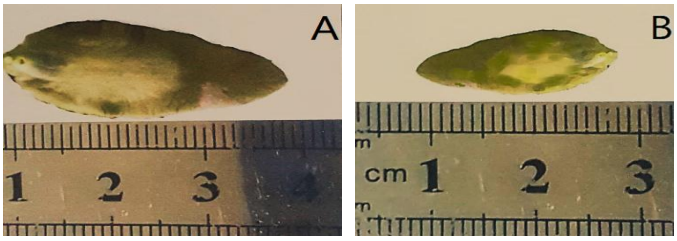

Figure 2. Sizes of *F. hepatica* parasite
A- Untreated B- Treatment

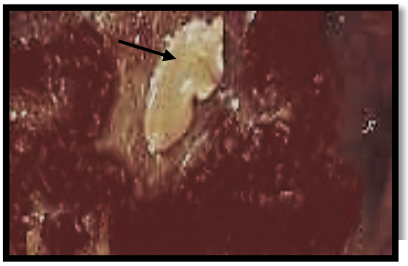

Figure 3. A goat liver infected with a parasite *F. hepatica*

4. Conclusion

This study concludes that goats were infected with *F. hepatica* in Anbar province with a high percentage. Consequently, The current study provides exploratory indicative data for future monitoring of parasitic diseases of medical importance, identifying risk factors and reducing economic loss to map strategies for controlling these diseases. Therefore, the veterinary authority must implement a control program to control these diseases by treating them using medicinal plants, improving the standard slaughterhouse system, and eliminating vector hosts.

References

[1] Pinilla, JC, Muñoz, AAF and Delgado, NU 2020, Prevalence and risk factors associated with liver fluke *Fasciola hepatica* in cattle and sheep in three municipalities in the Colombian Northeastern Mountains. *Veterinary Parasitology: Regional Studies and Reports*. **19**, 100364.

[2] Naranjo-Lucena, A, García, B, Campos A, Garza, Cuartero L, Britton L, Blanco A, Zintl, A and Mulcahy, G 2020, *Fasciola hepatica* products can alter the response of bovine immune cells to Mycobacterium avium subsp. paratuberculosis. *Parasite Immunology*, **42**(11), e12779.

[3] Shahatha, SS and Saleh, TA 2018, An Epidemiological, Diagnostic, and Therapeutic Study of the *Leishmania tropica* Parasite in Iraq's Anbar Province. *Baghdad Science Journal*, **15**(4), 392-400.

[4] Pereira, AE, Uribe, N and Pointier, JP 2020, Lymnaeidae from Santander and bordering departments of Colombia: Morphological characterization, molecular identification and natural infection with *Fasciola hepatica*. *Veterinary Parasitology: Regional Studies and Reports*, **20**, 100408.

[5] Ovchinnikov, VY, Kashina EV, Mordvinov, VA and Fromm, B 2020, EV-transported microRNAs of *Schistosoma mansoni* and *Fasciola hepatica*: Potential targets in definitive hosts. Infection, *Genetics and Evolution*, **85**,104528.

[6] Shahatha, SS 2019, An Epidemiological and Therapeutic Study of the *Cryptosporidium parvum* Parasite in Some Farm Animals in Anbar Province/Iraq. *Journal of Global Pharma Technology*, parasite. **10** (11):*147*-152.

[7] Hamoo, RN, Al-Rubaye FS, and Mustafa, NG 2020, Genotyping study of *Fasciola gigantica* isolated from cattle in Aqrah city, Iraq. *Iraqi Journal of Veterinary Sciences*, **34**(1), 123-127.

[8] Tran, DH and Phung, HTT 2020, Detecting *Fasciola hepatica* and *Fasciola gigantica* microRNAs with loop-mediated isothermal amplification (LAMP). *Journal Parasitic Disease*, **44**(2), 364-373.

[9] Achollah, AM, Karanja, DN and Bebora, LC 2020, Causes of organ condemnations in cattle at slaughter and associated financial losses in Siaya County, Kenya. *Journal Veterinary Medcine Animale Health*, **12**(2), 27-35.

[10] Abdulwahed, TK and Al-Amery, AM 2019, Morphological and molecular study of *Fasciola* spp. in sheep in Alkut city. *Internationl Journal Bioscience*, **14**(1), 121-130.

[11] Zafar, A, Khan MK, Sindhu D, Abbas RZ, Masood S, Abbas, Z and Javed, H 2019,

Seroprevalence of *Fasciola hepatica* in small ruminants of District Chakwal, Punjab, Pakistan. *Pakistan Veterinary Journal*, **39**(1), 96- 100.

[12] Hamami, H, Hamed, N and Ayadi, A 2007, Epidemiological studies on *Fasciola hepatica* in *Gafsa Oases (south west Tunisia)*. Parasite, **14**(3), 261-264.

[13] Khanjari, A, Bahonar A, Fallah S, Bagheri M, Alizadeh A, Fallah, M and Khanjari, Z 2014, Prevalence of fasciolosis and dicrocoeliosis in slaughtered sheep and goats in Amol Abattoir, Mazandaran, northern Iran. *Asian Pacific Journal of Tropical Disease*, **4**(2), 120-124.

[14] Abdul-Khader, NA 2002, Survey of cattle, sheep and goats infection with the liver worm *Fasciola hepatica* in Al-Qadisiyah governorate, *Al-Qadisiyah Journal of Veterinary Medcine Sciences*, **3**(1), 36-42.

[15] Mikaeel, FB 2020, Prevalence of *Fasciola hepatica* in Goats and Sheep by Using ELISA in Sera and Milk in Duhok, Iraq. *The Iraqi Journal of Veterinary Medcine*, **44**(2), 113-119.

[16] Al-khafaji, MA, Almremdhy, HAAE, Al-Sultany HHO and Abbas SM 2020, A retrospective survey with post mortem examination of liver fukes and lung hydatidosis in livestock in Babil, Iraq. *Plant Archives*, **20** (2). 4537-4543.

[17] Neamah, SI, Sarhan IA, Al-Shaye'a OA, 2018. Extraction and evaluation of the anti-inflammatory activity of six compounds of *Marrubium vulgare* L., *Bioscience Research*, **15**(3): 2393-2400.

[18] Shahatha, SS 2020, Epidemiological, diagnostic and therapeutic study for mange in sheep of Anbar province-Iraq. *Iraqi Journal of Veterinary Sciences*, **34**(1), 1-7.

[19] Blakelock, RA 1957, Notes on the Flora of 'Iraq with Keys: *Part IV. Kew Bulletin*, 461-497.

[20] Alvarez-Mercado, JM, Ibarra-Velarde F, Alonso-Díaz MÁ, VeraMontenegro Y, Avila-Acevedo, JG and García-Bores AM 2015, In vitro antihelmintic effect of fifteen tropical plant extracts on excysted flukes of *Fasciola hepatica*. *BMC veterinary research*, **11**(1),1-6.

[21] Ezeta-Miranda, A, Vera-Montenegro Y, Avila-Acevedo JG, García-Bores AM, Estrella-Parra EA, Francisco-Marquez, G and Ibarra-Velarde, F 2020, Efficacy of purified fractions of Artemisia ludoviciana Nutt. mexicana and ultraestructural damage to newly excysted juveniles of *Fasciola hepatica* in vitro. *Veterinary Parasitology*, **285**, 109184.

[22] Ghafar, A, Arbabi M, Mosayebi M, Hooshyar, H and Nickfarjam, AM 2021, Evaluation of anti-helmintic activity of Zingiber officinale roscoe extract on *Fasciola hepatica* miracidia In vitro. *International Archives of Health Sciences*, **8**(1): 45.

[23] Kendal, SB 1965, Relationship between the species of *Fasciola* and their molluscan hosts. In: Dawes B. (ed). In advances in parasitology. *Acadmaic press. London and Newyork*, **3**, 59-95.

[24] He, C, Ji X, Pan Y, Wang H, Wang K, Liang, M and Yang, L 2010, Antioxidant activity of alcoholic extract of Agrimonia pilosa Ledeb. *Medicinal chemistry research*, **19**(5), 448-461.

[25] Brusotti, G, Cesari I, Dentamaro A, Caccialanza, G and Massolini, G 2014, Isolation and characterization of bioactive compounds from plant resources: the role of analysis in the ethnopharmacological approach. *Journal of pharmaceutical and biomedical analysis*. **87**, 218-228.

[26] Jeyathilakan, N, Murali K, Anandaraj, A and Basith, SA 2012, In vitro evaluation of anthelmintic property of ethno-veterinary plant extracts against the liver fluke *Fasciola gigantica. Journal of Parasitic Diseases*, **36**(1), 26-30.

[27] SAS. Statistical Analysis system, 2012, users guide. *statistical version 9th ed. New York: SAS Institute Inc*; 5121 p.

[28] Salih, TA, Shahatha, SS and Alwan, A 2019, The Effect Study Of Adding Extracts Of Some Medicinal Plants In The Bloody, Serological And Histological Changes Of Eimeria Necatrix And Compared With Amprolium In Broiler Chickens Ross 308. *Plant Archives*, **19**(2), 1529-1537.

[29] Mohammed, NH 2020, Prevalence, morphological and biochemical study of larval stage Coenurus cerebralis of Taenia multiceps in sheep. *Iraqi Journal of Veterinary Sciences*

34(1), 159-163.

[30] Shahatha, SS 2019, Evaluation the efficiency of the fungus Metarhizium anisopliae as biocontrol agent for adults of hard ticks Hyalomma anatolicum. *Iraqi Journal of Veterinary Sciences*, **33**(2), 57-62.

[31] Shahatha, SS 2013, Prevalence of *Fasciola gigantica* among sheep in Al-Anbar govrnorate and study of effect for aqoues extract of Urtica urens L. on Viability of parasite. *Journal University of Anbar Pure sciences*, **7** (3), 33-38.

[32] Shahatha, SS 2019, An Epidemiological and Diagnostic Study of Anaplasma ovis Parasite in Native Goats in Anbar Province-Iraq. *International Journal of Drug Delivery Technology*, **9**(01), 34-38.

[33] Hassan, MM, Hoque MA, Islam SKMA, Khan SA, Roy, K and Banu, QA 2011, prevalence of parasites in black bengal goats in Chittagong, *Bangladesh. International Journal*, of Livestock Production, **2** (4), 40-44.

[34] Pérez-Creo, A, Diaz P, Lopez C, Béjar JP, Martínez-Sernández V, Panadero, R and Morrondo, P 2016, *Fasciola hepatica* in goats from north-western Spain: Risk factor analysis using a capture ELISA. *The Veterinary Journal*, **208**, 104-105.

[35] Shahatha, SS 2019, An Epidemiological, Diagnostic and Therapeutic Study of Giardia lamblia in Anbar Province–Iraq. *International Journal of Drug Delivery Technology*, **9**(01), 39-45.