Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7372 - 7379
Received 25 April 2021; Accepted 08 May 2021.

Estimating Serum Level of Human Monocyte Chemotactic Protien-1 (MCP-1) and Human Interferon Gamma Induced Protein 10 Kda (IP-10) in Patiants Infected with *Entamoeba Histolytica*

Maher Hussein Fadel[1] and Sukayna Jabbar Mushattat[2]

[1]University of Kufa, Faculty of Science, Iraq

[2]University of Kufa, Faculty of Science, Iraq

(E-mail: Sukaynaj.alhasnawee@uokufa.edu.iq; Mobile : 00964-7814544585)

Abstract

The current study was conducted to find out the effect of infection with *Entamoeba histolytica* on the level of concentration Human Monocyte Chemotactic Protien(MCP-1) and Human Interferon Gamma induced protrin 10 KDa(IP-10) in serum of the infected patiants. Blood samples were collected from the infected persons from AL- Najaf Alashrf city for the period from 1/9/2020 to 31/1/ 2021, The number of patients examined during the total study period reached 70 patients, while the number of control samples was 20. Verbal consent was taken from all individuals for inclusion in the study. The results of the examination showed a significant increase in the level of proteins MCP-1 & IP-10 in the blood serum of the infected persons compared with the control group, as well as different in the level of proteins according gender, as well as according to age groups and the area of residence at $P<0.05$.

Keywords: Human Monocyte Chemotactic Protien-1, MCP-1, Human Interferon Gamma, protein 10 Kda, IP-10

Introduction

Parasitic diseases pose a great challenge to health authorities in many countries, despite the great development that has occurred in the quality of medical services and methods for diagnosing, treating and controlling parasitic diseases[12], which in turn led to a clear reduction in the spread of these diseases in many industrialized and developed countries, The most important intestinal parasites pathogen for humans are the *E. histolytica*,Amoebiasis, or amoebic dysentery, is a term used to describe an infection caused by the protozoan *E. histolytica* [1]. symptoms of amoebiasis include watery or bloody diarrhea, crampinn, abdominal pain, weight loss and colitis, diarrheal disease second wordwide ranks after pneumonia as the main cause in children under the age of five, and intestinal amoebiasis is one of the main factors causing severe diarrhea in the developing world[2]. there are other symptoms in this case such as coughing, hepatomegaly, elevated alkaline an leukocytosis, Approximately 90% of *E.histolytica* asymptomatic, But it is more common in an injury to the intestine[3]. Immune response against the parasite, the infected cell produce NO and chemokines for attract immune cells [4]. One of the most important response of infected people is the generation of specific IgG and IgA antibodies,In recent times, many studies have focused on hormonal and enzymatic regulation and the immune response directed against pathogens upon infection, and this is evident from various parasitic diseases [5].

Materials and Methods

Individual samples

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7372 - 7379
Received 25 April 2021; Accepted 08 May 2021.

The current study was conducted in the AL- Najaf Alashrf city for the the period from 1/9/2020 to 31/1/ 2021. The study included patients with *Entamoeba histolytica* who referred internal medicine doctors to hospitals Al-Hakim, Furat, and Sajjad to be examined clinically.The number of patients examined during the total study period reached 70 patients, while the number of control samples was 20. Verbal consent was taken from all individuals for inclusion in the study[6].

Serum Collection

After the stool sample examination, if it is found that there are cysts or trophozoites *Entamoeba histolytica*,, a blood sample is drawn from the patient through a medical syringe and in an amount of 3-5 ml ,a tube gel is placed and left for 10-20 minutes at room temperature for the blood to clot, and the tube should not be moved to prevent hemolysis, then it is placed in a centrifuge for a period of 15-20 minutes at a speed of 3000 RPM to separate the serum from the coagulant material, so the coagulant material precipitates at the bottom of the tube and the serum remains at the top of the tube and its color is usually yellow, after that, the serum is taken by pipettes and placed in **E**ppendorf's tubes of 100 microliters, and each sample of serum is divided into 4 **E**ppendorf's tubes, and then we take the required information from the patient according to the form shown in Table 3-3 , then each sample is label and placed in samples portfolio and kept in the refrigerator at -20C to be frozen until the laboratory analyzes required in the study are carried ou[7]t.

Human interferon gamma induced protein 10kDa (IP-10) measurement

Determination of IP-10 protein by ELISA Kit, using the ELISA device
IP-10 protein was measured from serum samples of patients using kit materials (IP-10 ELISA Kit) supplied by Bioassay Technology Laboratory (BT LAB) company[8].

Human monocyte chemotactic protein 1 (MCP-1) measurement

Determination of MCP-1protein by ELISA Kit, using the ELISA device, IP-10 protein was measured from serum samples of patients using kit materials (IP-10 ELISA Kit) supplied by Bioassay Technology Laboratory (BT LAB) company.

Analysis of data: The data are analyzed through the application of descriptive and inferential statistical analysis procedures and the data income through SPSS. - 23[9].

Results

Measurement of IP-10 concentration according gender of samples of patients with *E.histolytica* infection

The comparison between females and males with *E.histolytica* infection for the rate of IP-10 concentration were significant increase ($p < 0.05$) (135 ± 104 ng / l), (112 ± 128.5 ng / l), respectively, for the level of IP-10 concentration in females over males, as seen in Table 1 and Figure 1.

Table 1: Shows rate concentration of IP-10 in blood serum comparison between females and males suffering from *E.histolytica* infection.

Gender	Concentration of IP-10 (ng / l) M±SD
Males	112 ± 128.5
Females	*135 ± 104
Tc	**7.8**
Tt p < 0.05	**1.98**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7372 - 7379
Received 25 April 2021; Accepted 08 May 2021.

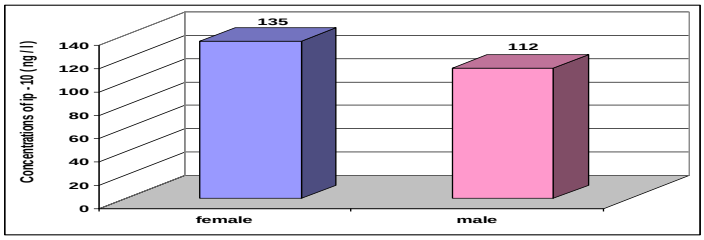

Figure 1:Shows percentages of rate concentration of IP-10 in blood serum comparison between females and males suffering from *E.histolytica* infection.

2- Measurement of IP-10 concentration according age groups of samples

Age groups infection were compared to the level of IP-10 concentration, and it was found in 31-40 and 51-60 years (262 ± 148 ng / l), (238 ± 200 ng / l), respectively, and a significant decrease in the age group 1-10 years (64 ± 8.4 ng / l), at P<0.05, as seen in Table 2 and Figure 2.

Table 2 **: Shows rate concentration of IP-10 in blood serum comparison between age groups suffering from *E.histolytica* infection.**

Categories (year)	**Concentration of IP-10 (ng / l) M±SD**
1 – 10	**64 ± 8.4**
11 – 20	**67± 14.3**
21 – 30	**86 ± 39.8**
31 – 40	****262 ± 148**
41 – 50	**182 ± 81**
51 – 60	***238 ± 200**
61 – 70	**165 ± 143.6**
LSD P< 0.05	**21.5**

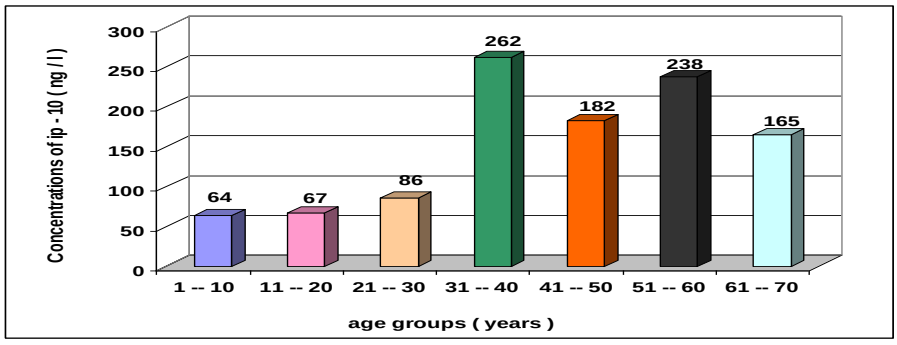

Figure 2**:Shows percentages of rate concentration of IP-10 in blood serum comparison between age groups suffering from *E.histolytica* infection**.

Measurement of IP-10 concentration according area of residence for samples of patients with *E.histolytica* infection

The comparison between rural areas and urban areas with *E.histolytica* infection for the rate of IP-10 concentration were significant increase (p < 0.05) (151 ± 136.8ng / l), (103 ± 109.5 ng / l), respectively, for the level of IP-10 concentration in rural areas over urban areas, as seen in Table 3. and Figure 3.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7372 - 7379
Received 25 April 2021; Accepted 08 May 2021.

Table 3: Shows rate concentration of IP-10 in blood serum in rural and urban areas for patients with *E.histolytica* infection.

Residential address	Concentration of IP-10 (ng / l) M±SD
Rural	***151 ± 136.8**
Urban	**103 ± 109.5**
Tc	**8.8**
Tt p < 0.05	**1.98**

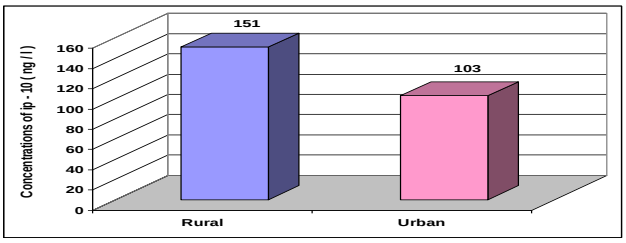

Figure 3:Shows percentages of concentration of IP-10 in blood serum in rural and urban areas for patients with *E.histolytica* infection.

Measurement human monocyte chemotactic protein 1 (MCP-1) in serum

The current study revealed that concentration of MCP-1in infected patients with *E. histolytica* were significant increase ($p < 0.05$) (212.5 ± 134 ng / l) in compares to the control group (202.5 ± 78 ng / l), as seen in Table 4 and Figure 4.

Table 4: Shows rate concentration of MCP-1in blood serum comparison between patients suffering from *E.histolytica* infection and control group.

Type of sample	Concentration of MCP-1(ng / l) M±SD
patients	*212.5 ± 134
control	202.5 ± 78
Tc	**2.6**
Tt p < 0.05	**1.98**

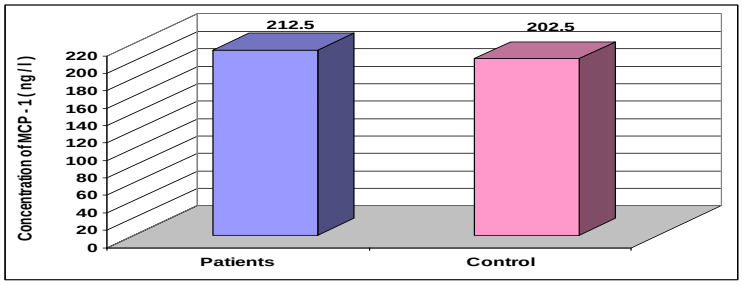

Figure 4:Shows percentages of rate concentration of MCP-1 in blood serum comparison between patients suffering from *E.histolytica* infection and control group.

Measurement of MCP-1concentration according gender of samples of patients with *E.histolytica* infection

The comparison between males and females with *E.histolytica* infection for the rate of MCP-1concentration it turns out there is no significant difference ($p < 0.05$) (210 ± 178 ng /

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7372 - 7379
Received 25 April 2021; Accepted 08 May 2021.

l), (215 ± 89.8 ng / l), respectively, for the level of MCP-1concentration in females and males, as seen in Table 5 and Figure 5.

Table 5: Shows rate concentration of MCP-1in blood serum comparison between females and males suffering from *E.histolytica* infection.

Gender	Concentration of MCP-1(ng / l) M±SD
Males	210 ± 178
Females	215 ± 89.8
Tc	**1.1 non sign**
Tt p < 0.05	**1.98**

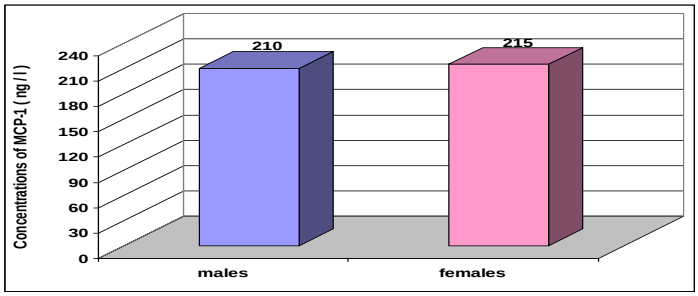

Figure 5:Shows percentages of rate concentration of MCP-1in blood serum comparison between females and males suffering from *E.histolytica* infection.

Measurement of MCP-1concentration according age groups of samples

Age groups infection were compared to the level of IP-10 concentration, and it was found in group 51-60 and 51-60 years (408 ± 254 ng / l), and a significant decrease in the age group 1-10 years (135 ± 13.5 ng / l), as seen in Table 6and Figure 6.

Table 6:Shows rate concentration of MCP-1 in blood serum comparison between age groups suffering from *E.histolytica* infection.

Categories (year)	**Concentration of MCP-1(ng / l) M±SD**
1 – 10	**135 ± 13.5**
11 – 20	**140 ± 19.5**
21 – 30	**153± 55.3**
31 – 40	**144 ± 30.7**
41 – 50	**272 ± 264**
51 – 60	***408 ± 254**
61 – 70	**181± 12.8**
LSD P< 0.05	**36.4**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7372 - 7379
Received 25 April 2021; Accepted 08 May 2021.

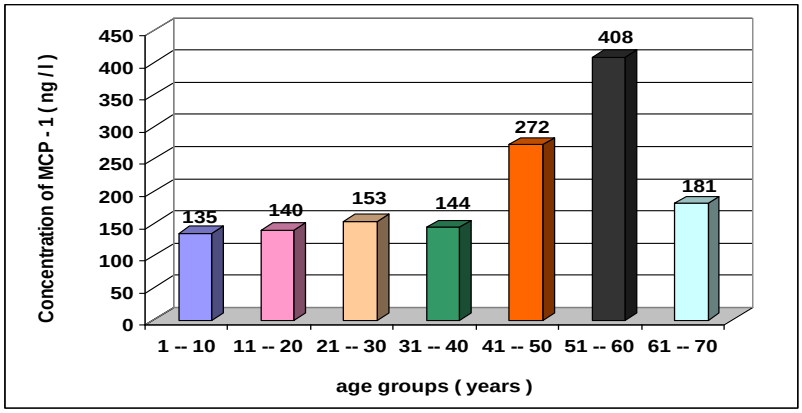

Figure 6:Shows percentages of rate concentration of MCP-1 in blood serum comparison between age groups suffering from *E.histolytica* infection.

Measurement of MCP-1concentration according area of residence for samples of patients with *E.histolytica* infection

The comparison between rural areas and urban areas with *E.histolytica* infection for the rate of MCP-1concentration were significant increase ($p < 0.05$) (239 ± 161.7 ng / l), (198 ± 149.4 ng / l), respectively, for the level of MCP-1 concentration in rural areas over urban areas, as seen in Table 7 and Figure 7.

Table 7: Shows rate concentration of MCP-1in blood serum in rural and urban areas for patients with *E.histolytica* infection.

Residential address	Concentration of MCP-1(ng / l) M±SD
Rural	***239 ± 161.7**
Urban	**198 ± 149.4**
Tc	**12.9**
Tt p < 0.05	**1.98**

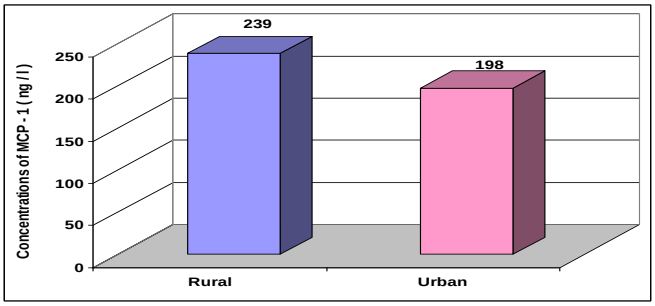

Figure 7:Shows percentages of rate concentration of MCP-1in blood serum in rural and urban areas for patients with *E.histolytica* infection.

Discussion

The current result of IP-10 of infected patients (123.5 ± 116 ng / l) compares to the control group (97.5 ± 90 ng / l), the systemic levels of IP-10 are significantly increased in IBD patients when compared to normal healthy donors, moreover, IP-10 expression is also increased in the mucosa of IBD patients [7], IP-10 has an important role in IBD and adaptive mucosal immunity and inflammation, , also, a significant association between increased IP-10 and the severity of parasitic diseases has been shown When comparing between females and

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7372 - 7379
Received 25 April 2021; Accepted 08 May 2021.

males with infected *E.histolytica*, an increase in the rate of IP-10 concentration was found in females (135 ± 104 ng / l) than in males (112 ± 128.5 ng / l) [11] , There are influences of gender on all aspects of generation, and the effects of cytokines and immune chemokines on normal adaptive immune responses, and autoimmune diseases, this suggests that perhaps host defense infection problems are stronger drivers of increased IP-10 production than immune in conditions of inflammatory in women, not men, sex hormones may affect the increase or decrease of IP-10 concentration [10]. The age groups of patients with *E.histolytica* infection were compared to the level of IP-10 concentration, and it was found in the age groups 31-40 and 51-60 years (262 ± 148 ng / l), (238 ± 200 ng / l), respectively, and a significant decrease in the age group 1-10 years (64 ± 8.4 ng / l), This increase in the concentration of IP-10 is associated with immune or inflammatory diseases of the elderly, When comparing rural and urban areas with infected *E.histolytica* for IP-10 concentration rate, the levels of IP-10 were found to be higher in person in rural areas (151 ± 136.8ng / l) compared to urban areas (103 ± 109.5 ng / l) [14] ,Person in rural areas are often more susceptible to infection with parasites in general and *E.histolytica* in particular for many reason including a lack of health awareness, lack of interest in hygiene, as well as a decreased immune response, and climatic and economic variation, and sociocultural condition, thus increasing the inflammatory diseases caused by parasites, and thus a higher concentration of IP-10 in the serum for the treatment of intestinal infection[18]. The current study revealed that the concentration of MCP-1 in *E.histolytica* patients was increased (212.5 ± 134 ng / l) compared to the control group (202.5 ± 78 ng / l) [16]. MCP-1 produced by many cell types, including endothelial, fibroblasts, epithelial and smooth muscle, however, monocyte/macrophages are found to be the major source of MCP-1[12] demonstrate that the soluble proteins secreted by *E.histolytica* synthesizes its own prostaglandin E2 (PGE2) via a novel cyclooxygenase- like enzyme, PGE2 is endogenously synthesized and present in secretory soluble components of amoeba, it can induce IL-8 production in colonic epithelial cell, and this cause release of epithelium cytokines/chemokines [13]. parasite release of inhibitory factors, , indicating the role of MCP-1 in severity of IBD[19].no significant difference, as the MCP-1 concentration was in males (210 ± 178 ng / l), and in females (215 ± 89.8 ng / l), the level of MCP-1 concentration increase in the age group 51-60 years (408 ± 254 ng / l), and a decrease in the age group 1-10 years (135 ± 13.5 ng / l) [17].

References

1. Abdelseed , N. A. H. . (2021). Glycemic Control and Knowledge among Children and Adolescents with Type 1 Diabetes Mellitus: A Cross-sectional Study . Journal of Scientific Research in Medical and Biological Sciences, 2(1), 1-9. https://doi.org/10.47631/jsrmbs.v2i1.149
2. Pandey , B. N.; Kumar, D. ; Verma D. K. (2013). Epidemiological study of parasitic infestations in rural women of Terai belt of Bihar, India. Ann. Bio. Res.; 4 (10):30-33.
3. 2-Al-Mozan, H. D. K., Daoud, Y. T., & Dakhil, K. M. (2018). Effect of some factors on intestinal parasitic infections. *University of Thi-Qar Journal of Agricultural Research*, *7*(1), 361–376.
4. 3-Ngobeni R, Samie A, Moonah S. Entamoeba in South Africa: correlations with the host microbiome, parasite burden and first description of *E. bangladeshi* outside of Asia. J Infect Dis 2017; 216:1592–1600.
5. 4-Bazzaz, A. A., Shakir, O. M., & Alabbasy, R. H. (2017). Prevalence of two gastrointestinal parasites Entamoeba histolytica and Giardia lamblia within Samarra city, Iraq. *Advances in Bioscience and Biotechnology*, *8*(11), 399–410.
6. 5-Sofa Dugue-Beltran; Ruben Santago- Orejuela; Adriana Arevalo-Jamaica; Rafael Guerrero- Lozano; Jonia Montenalis (2002) Antigen in human fecal eluates by Enzy me- linked immunosorbent assay using polyclonal Antibodies. Univ. H. Sc. Ce., New Orleans. 97(8): 1165-1168 .

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 7372 - 7379
Received 25 April 2021; Accepted 08 May 2021.

7. Klein, SL. (2000).Hormones and mating system affect sex and species differences in immune function among vertebrates. Behavioural Processes. 51:149-166.
8. Blaise, C. (2007). Round trip Ticket for Secretory IgA: Role in Mucosal Homeostasis. J. Immunol,; 178 (1): 27-32.
9. 8-Al- Kubassi, ABH.(2002). Immunological , epidemiological study of patients infected with Entamoeba histolytica Ph.D. Thesis,Coll. Sci. Allmustan. Univ . p 116-118.
10. Sukayna, J. M.; Saleem, K. A.; Jabbar A.M.(2018). Effect of Magnetized Water on Reducing the Histological and Physiological Effects of Experimental Infection with *Ascaridia galli* in Domestic Chicken. JGPT. ISSN: 0975 -8542. 2018; 10(01):97-103.
11. Ahmed, A. J. A.; Saleem,A.; Sukayna, J.M.(2018). Immune Response in Pregnant Women Infected with Acute Vaginal Abscess Caused by Staphylococcus Aureus and Trichomonas Vaginalis. JCDR/2018/35653.11643, 2018 Jun, Vol-12(6): DC51-DC55.
12. Sukayna, J. M. & Jabbar, A. A.(2018). Effect Addition of the Extract *Nigella sativa* on the Histological and Physiological Changes of the Domestic Chicken Experimental Infected with *Eimeria maxima.* J. Pharm. Sci. & Res. Vol. 10(8), 2018, 1934-1938.
13. Jabbar, E. M.& Noor, M. H.(2018). The Effect of Cirprofloxacin (CPX) on the Histological Structure of Albino Rabbit Ovary. Journal of Global Pharma Technology| 2018; 10(03):498-508.
14. Jabbar, A. M. Al.& Methak, A. A.(2016). The Histological structure of Thyroid gland and the relationship between the hyperthyroidism and totalprotein,albumin, globulin, liver enzymes and some minerals deficiency. IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563Vol.9, No.8, pp 189-196, 2016.
15. 14- Thafar, N. A.; Arshad, N.A.;, Jabbar, A. A.(2016). Effect of Ethanolic Extracts of *Salvadora persica* Roots on Female Albino Rats. ISSN: 0975-8585 RJPBCS 7(6) Page No. 1115.
16. Hawraa f. Al-Baghdad and Mohammed,A.Jabbar(2019).Study of histological and embryonic chang in chicken embryos treated with hot water ginger extract.forensic medicine &toxicology.
17. Ensaf,Saleh Abar and Jabbar Abadi Alaridhi(2019). STUDY OF THE EFFECT OF AQUEOUS EXTRACT OF (GINGER) *ZINGIBEROFFICINALE ROSCO* IN THE HISTOLOGICAL STRUCTURE OF PROSTATE GLAND OF WHITE MALE RABBITS *ORYCTOLAGUS CUNICULUS. Plant Archives* Vol. 19, Supplement 1, 2019 pp. 293-298.
18. Mushattat,S.J.&Alaridi,J.A.(2018). Effect of cold water extract Zingiber *officinale* on the Histological changes of the Experimental infection of domestic chickens with *Ascaridia galii.* Journal of Pharmaceutical Sciences and Research. Vol. 12(1), 2020, 186-190.
19. Hawraa f. Al-Baghdad and Mohammed,A.Jabbar(2019).Study of histological and embryonic chang in chicken embryos treated with hot water ginger extract.forensic medicine &toxicology.
20. Hassan,A.K. & Mohammed,J.A. (2020).The study 0f side effect of levanofloxacin on histological structuerof brain in white rats mal. SYLWAN journal ,Vol. 164(5), 2020, 186-190.