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RESEARCH ARTICLE

Effects *Cuminum cyminum* Extracts in Lipid Profile Levels and Internal Tissues of Infected Balb/c Mice with Hydatid Cysts

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Abstract

Hydatidosis is one of the most cosmopolitan zoonotic disease that may causes death and still very common in the developing world. The present study aimed to detect the effects of Albendazole and *C. cyminum* extracts on lipid profile, liver and spleen of infected Balb/c mice with hydatidosis. This study showed positive results for tannins, phenolic compounds, flavonoids and glycosides in alcoholic and aqueous extracts of *C. cyminum*, while negative result for alkaloids. Significant ($p \leq 0.05$) increasing of total cholesterol was detected for the third (273.57 mg/dl) and fourth (281.73 mg/dl) positive control groups. Triglycerides shows significant increasing in groups 3 (248.65 mg/dl) and 4 (260.30 mg/dl) of positive control groups. About HDL-C, significant decreasing was recorded in the third and fourth positive control groups (40.87 mg/dl and 33.42 mg/dl, respectively), while significant ($p \leq 0.05$) elevation was occurred for HDL-C in Albendazole treatment group (60.87 mg/dl) as compared with the negative control group, with no significant differences were reported for HDL-C in alcohol and aqueous extract treated groups. For histological study, the present study showed degeneration of hepatocytes, thickening wall of hepatic blood vessels and infiltration of lymphocytes for Albendazole treatment group, while alcoholic and aqueous extract treated groups showed central vein, hepatocytes and sinusoids with normal sizes. Concerning spleen, results in the current study showed normal shape and size for white pulp, red pulp and central artery of all treatment groups, while edema and necrotic materials appeared in some of positive control groups.

Keywords: *Hydatidosis, Hydatid cyst, Cuminum cyminum extract Histological effects.*

Introduction

Echinococcus granulosus is a tapeworm belong family Taeniidae that causes hydatidosis or cystic echinococcosis in human and most of herbivorous animals [1]. The life cycle of *E. granulosus* needs two mammalian hosts, including an intermediate host of herbivorous animal and definitive canine host (dogs), while human is considered as an accidental host [2].

Hydatidosis is one of the most cosmopolitan zoonotic disease that may causes death and still very common in the developing world [3]. The internal organs especially liver and lungs of humans and all intermediate hosts are the main sites for hydatid cyst, which appears like unilocular fluid-filled bladder [4]. Cystic echinococcosis causes pathological damages or dysfunction especially by the gradual effecting of space-occupying repression or displacement of vital host tissue, vessels or organs [5]. The symptoms depend on the habitat, number and size of the cysts and the complications caused by rupture of cysts [6]. Albendazole is a useful drug for the treatment of hydatidosis [7]. In the last years, some of medical plants are used as alternative treatment instead of the artificial chemical drugs, because it has active anti-microbial properties due to existence of active compounds.

The active compounds are produced due to secondary metabolism in the plants, these compounds including flavonoids, glycosides and phenolic compounds [8]. Cuminum cyminum is one of medical plants belong family Aplaceae that using for digestive system disorders, intestinal helminths eviction and sterilizing of urinary tract [9]. The present study aimed to detect the effects of Albendazole and C. cyminum extracts

(alcoholic and aqueous) on lipid profile, liver and spleen of infected Balb/c mice with hydatidosis.

Materials and Methods

Plant Extracts

C. cyminum was obtained from College of Agriculture, Tikrit University, Iraq and it was identified by the National Herbarium of Iraq, Ministry of Agriculture Add paragraph about.

Plant Extracts Preparation

Mice were obtained from National Center for Drug Control and Research (NCDCR) in Baghdad, Iraq. This study was performed from February of 2016 to June of 2016

Phytochemicals Constituents

Alcoholic and aqueous extracts of *C. cyminum*, Dragendroff's test was used to detect Alkaloids, and appearance of red sediment means a positive result [19, 20]. Tannins and phenolic compounds were determined by ferric chloride test, dark blue and blackish green colors are the evidences for the positive result [21], while appearance of black or blackish red color when ferric chloride test used that the evidence for existing flavonoids [22]. Sodium hydroxide test was used to detect glycosides and yellow color produces for the positive result [23].

Isolation of *E. granulosus* Protoscoleces

Protoscoleces of *E. granulosus* were isolated from ovine fertile hydatid cysts and conserved it in the 1:4 (v/v) of hydatid cyst fluid and Krep's Ringer solution According to [10]. Krep's Ringer solution was prepared Based on [11].Viability and number of protoscoleces were done according to [12] using 0.1% of Eosin stain.

Experimental Design

A total of 40 Balb/c mice with age 12-14 weeks and weight 30-35 gm were divided in to 8 groups included 5 mice per group:

- Negative control group that injected with phosphate buffer saline (PBS) in the peritoneal cavity [13, 14]. PBS was prepared according to [15] during the use.
- Positive control groups (4) which injected with 2500 protoscoleces that suspended in 1 ml of PBS by syringe 3 ml and needle 23G, then dissected and examined after 1, 2, 4 and 6 weeks.
- Treatment groups three groups were infected experimentally first group treated with 15 mg/ kg / body weight daily of Albendazole [16]. Second and third groups were dosed with 500 mg/ kg / body weight daily of alcoholic extract of *C. cyminum* that prepared based on [17] and aqueous extract of *C. cyminum*, which prepared according to [18], respectively. Treatment was continued for 6 weeks, Iraq All.

Lipid Profile

In the present study, total serum cholesterol, serum triglycerides and heavy density lipoprotein-C (HDL-C) were estimated using three commercial kits (Randox, UK) according to the manufacturer's instructions.

Histological Tests

Histological sections of mice liver and spleen were prepared according to [24] from all mice groups in the present study.

Statistical Analysis

Duncan's Multiple Range Test (DMRT) was used to analyze the data statistically with SPSS software program [25].

Results and Discussion

Active Compounds of *C. cyminum* Extracts

The present study showed positive results for tannins, phenolic compounds, flavonoids and glycosides in alcoholic and aqueous extracts of *C. cyminum*, while negative result for alkaloids (Table 1).

Table 1: Active compounds in alcoholic and aqueous extracts of *C. cyminum*

Active compounds	Type of extract	
	Alcoholic extract	Aqueous extract
Tannins	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Glycosides	+	+
Alkaloids	-	-

The current study agreed with study of [26] that detected tannins and glycosides in *C. cyminum* extracts and no alkaloids were found. Also, our study agreed with [27] that found flavonoids and glycosides in *C. cyminum* extracts. The present study agreed with study of [28] for existence of tannins, phenolic compounds, flavonoids and glycosides in *C. cyminum* extracts. The chemical detection for active compounds in *C. cyminum* extracts showed antioxidant activity, enzymatic control, growth inhibition for many organisms included parasites, fungi and bacteria [29, 30, 31]. These active compounds have growth inhibition activity for many parasites that involved *E. granulosus* [29, 32].

Lipid Profile

Results for total serum cholesterol, serum triglycerides and heavy density lipoprotein-C (HDL-C) of the present study were showed in Table 2.

Total Serum Cholesterol

Non-significant variances were recorded for total serum cholesterol between first and second groups of positive control (236.94 mg/dl and 222.29 mg/dl, respectively) with the negative control group 203.30 mg/dl, while, significant ($p \leq 0.05$) increasing was detected for the third (273.57 mg/dl) and fourth (281.73 mg/dl) positive control groups when compared with negative control.

Also, non-significant differences were reported for total serum cholesterol among all treatment groups (Albendazole 219.34 mg/dl, alcoholic extract 245.74 mg/dl and aqueous extract 237.07 mg/dl) as compared with the negative control group. The rising of total serum cholesterol might due to decrease for HDL-C that responsible in the transporting of cholesterol from somatic cells to hepatic via blood, therefor cholesterol accumulated in the blood [33].

Triglycerides

For positive control groups, non-significant variances were recorded between groups 1 and 2 (194.65 and 203.90 mg/dl, respectively), while significant increasing for groups 3 (248.65 mg/dl) and 4 (260.30 mg/dl) when compared with the negative control group (178.00 mg/dl). In addition, there are no significant variances for triglycerides among whole treatment groups; Albendazole treatment group, alcoholic and aqueous extract treated groups (169.54 mg/dl, 198.44 mg/dl and 176.67 mg/dl, respectively) as comparison with the negative control group.

These results due to existence of phenolic compounds, flavonoids and glycosides, which inhabit the reactions of free radicals formation via removing these radicals via antioxidant activity, increasing of antioxidant hepatic enzymes activity such as catalase and superoxide dismutase [34]. In addition, these active compounds increase insulin sensitivity that increases lipoprotein lipase activity, the last can be analyzing triglycerides to fatty acids and glycerol [35].

Heavy Density Lipoprotein-C (HDL-C)

In the present study, the positive control groups showed non-significant variances in the first (48.02 mg/dl) and second (49.82 mg/dl) groups, while significant decreasing was happened in the third and fourth positive control groups (40.87 mg/dl and 33.42 mg/dl, respectively) as comparison with the negative control group (47.63 mg/dl). For treatment groups, significant ($p \leq 0.05$) increasing was occurred for HDL-C in Albendazole treatment group (60.87 mg/dl) as compared with the negative control group, while no significant differences were reported for HDL-C in alcohol and aqueous extract treated groups when compared with the negative control group.

Many reasons for HDL-C decreasing; hepatic lipase effected by metabolic disorders, which may cause by parasite and lead to break HDL-C molecule, then take cholesterol from hepatocytes for transforming it to bile salts. Another reason may explains HDL-C decreasing, the lower activity of lysis for very low density lipoprotein (VLDL) and functional changes in liver that caused by hydatidosis [36, 37].

Table 2: lipid profile of treatment, positive control and negative control groups

Parameters / Groups	Total Cholesterol Mean± SD (mg/dl)	Triglycerides Mean± SD (mg/dl)	HDL-C Mean± SD (mg/dl)
Albend. Treat.	219.34 ± 95.83 AB	169.54 ± 29.27 C	60.87 ± 21.73 A
Alco. Ext. Treat.	245.74 ± 61.49 AB.	198.44 ± 57.88 C	42.86 ± 17.78 BC
Aque. Ext. Treat.	237.07 ± 55.81 AB	176.67 ± 60.67 C	41.28 ± 12.53 BC

+ve Ctrl. 1	236.94 ± 65.08 AB	194.65 ± 39.53 C	48.02 ± 10.16 AB
+ve Ctrl. 2	222.29 ± 58.72 AB	203.90 ± 30.20 BC	49.82 ± 9.81 AB
+ve Ctrl. 3	273.57 ± 68.30 A	248.65 ± 39.68 AB	40.78 ± 9.95 BC
+ve Ctrl. 4	281.73 ± 88.31 A	260.30 ± 58.93 A	33.42 ± 5.79 C
-ve Ctrl.	203.30 ± 40.62 B	178.00 ± 17.22 C	47.63 ± 8.87 B

Histological study

Liver

The present study showed degeneration of hepatocytes, thickening wall of blood vessels and infiltration of lymphocytes for Albendazole treatment group (Figure1). These changes may be relating to immunity and presence of hepatic hydatid cyst. In addition, Albendazole causes infiltration for immune cells [38, 39], while the toxic substances that secreted by parasite cause degeneration through autolysis of hepatocytes [40].

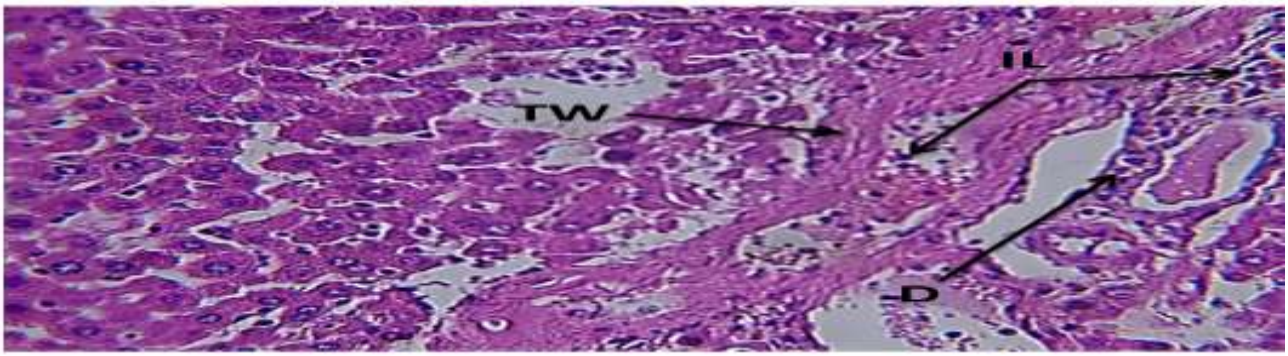

Figure1: Hepatic section of Albendazole treatment group shows degeneration of hepatocytes (D), thickening wall of blood vessels (TW) and infiltration of lymphocytes (IL), H and E400x

For alcoholic and aqueous extract treated groups (Figures 2 and 3, respectively), hepatic sections showed central vein, hepatocytes and sinusoids with normal sizes. The reason for positive effect of *C. cyminum* extracts on hepatic tissue is presence of antioxidant compounds such as flavonoids and phenolic compounds that protect liver from parasitic toxins, anti-lipid peroxidation and inhibit lysis of hepatocytes membrane [41, 42, 43].

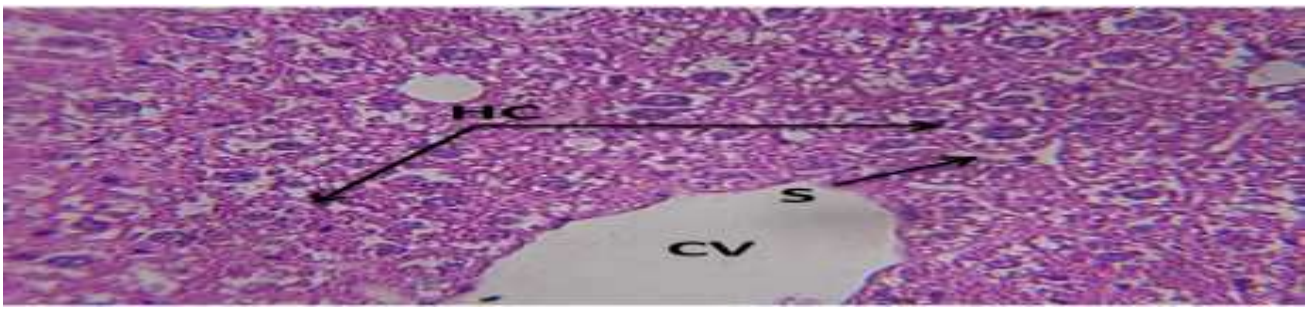

Figure 2: Hepatic section of alcoholic group of *C. cyminum* shows central vein (CV), hepatocytes (HC) and sinusoids (S) with normal sizes, H and E 400 xs

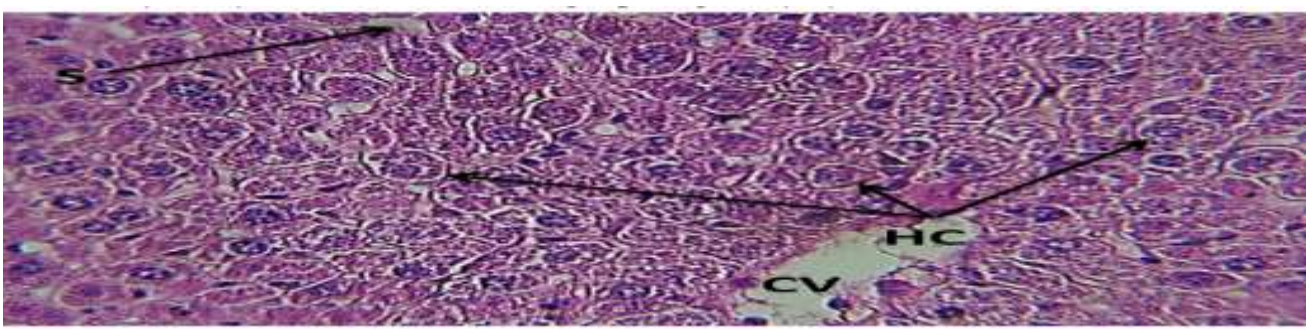

Figure 3: Hepatic section of aqueous group of *C. cyminum* shows central vein (CV) hepatocytes (HC) and sinusoids (S) with normal sizes, H and E 400 xs

For positive control groups, the present study showed congestion of blood vessels and infiltration of lymphocytes in third and fourth positive control groups (Figures 4 and 5, respectively) as compared with negative control group (Figure 6) that showed normal shape for central vein, hepatocytes and sinusoids.

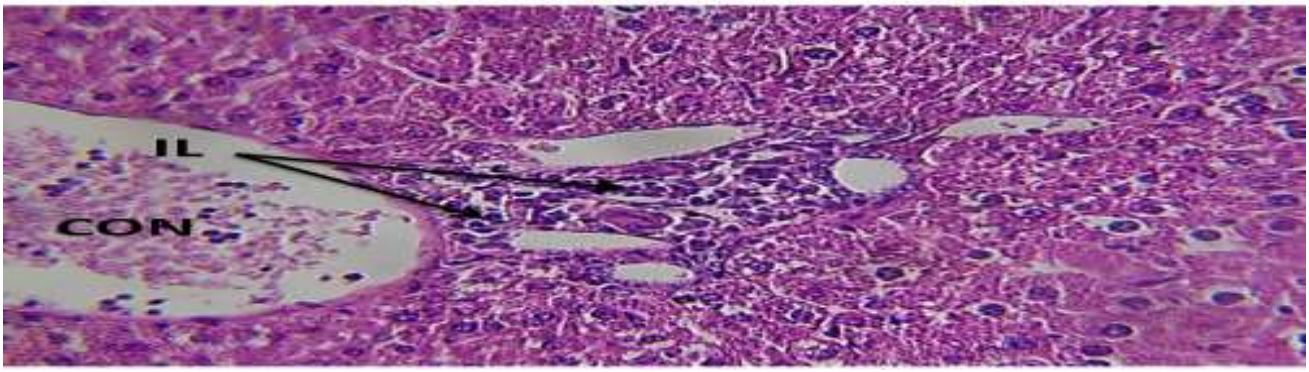

Figure 4: Hepatic section for third group of positive control shows congestion of blood vessels (CON) and infiltration of lymphocytes (IL), H and E 400x

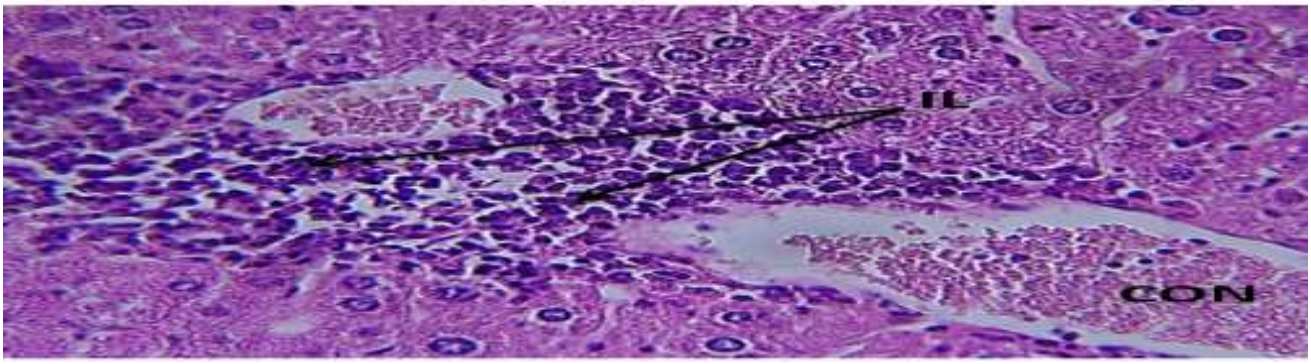

Figure 5: Hepatic section for fourth group of positive control shows congestion of blood vessels (CON) and infiltration of lymphocytes (IL), H and E 400x

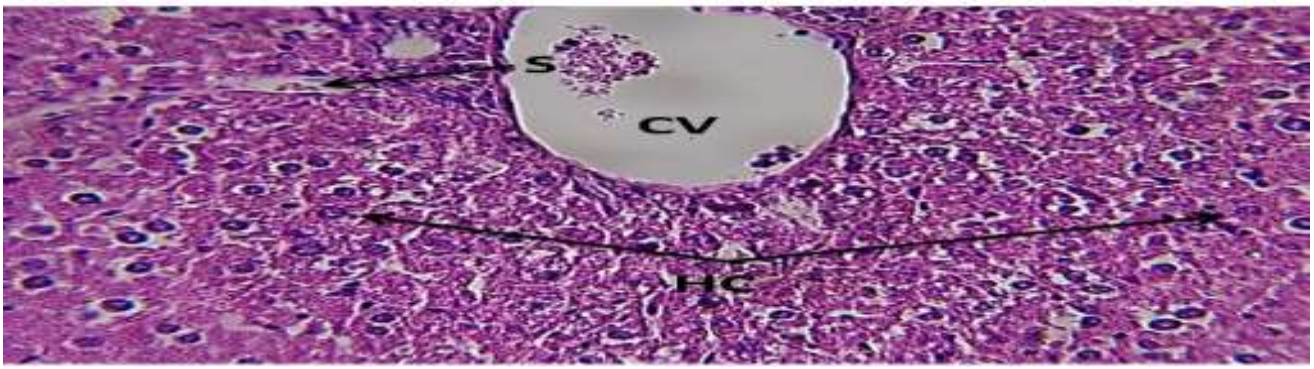

Figure 6: Hepatic section of negative control group shows central vein (CV), hepatocytes (HC) and sinusoids (S) with normal shape, H and E 400 xs

The present study agreed with previous studies [44, 45] that detected lymphocytes infiltration and blood vessels congestion associated with hydatid cyst infection. Hossain et al., mentioned that histopathological effects occur because hydatidosis infection [46]. Polloca et al. Referred to the infiltration in the location of infection, which attributed to parasitic toxins that lead to eosinophils activation [47]. While, other study reported congestion of blood vessels due to weakness in the blood discharge as result to occlusion of hepatic veins by hydatid cyst pressure [48].

Spleen

Results in the current study showed normal shape and size for white pulp, red pulp and central artery of all treatment groups (Figures 7, 8 and 9). The main reason for these results is presence of antioxidant compounds in *C. cyminum* extracts, which prevent the binding between hydroxyl free radical with proteins or amino acids [49]. In addition, these active compounds protect DNA of splenic cells and prevent the oxidative stress [50].

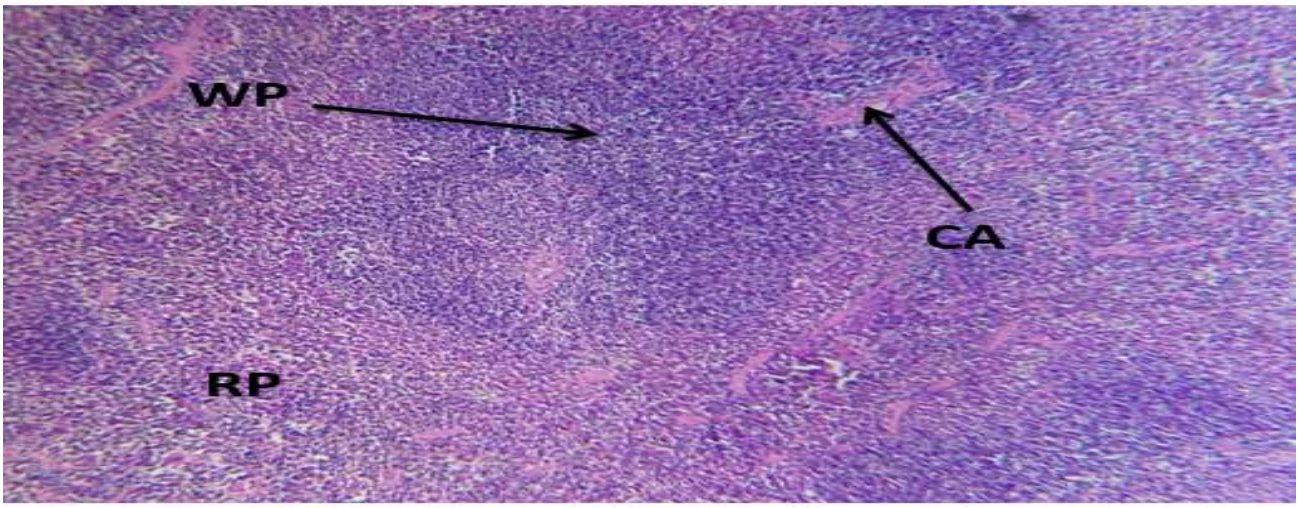

Figure 7: Splenic section of Albendazole treatment group shows a normal tissue for white pulp (WP), red pulp (RP) and central artery (CA), H and E 100x

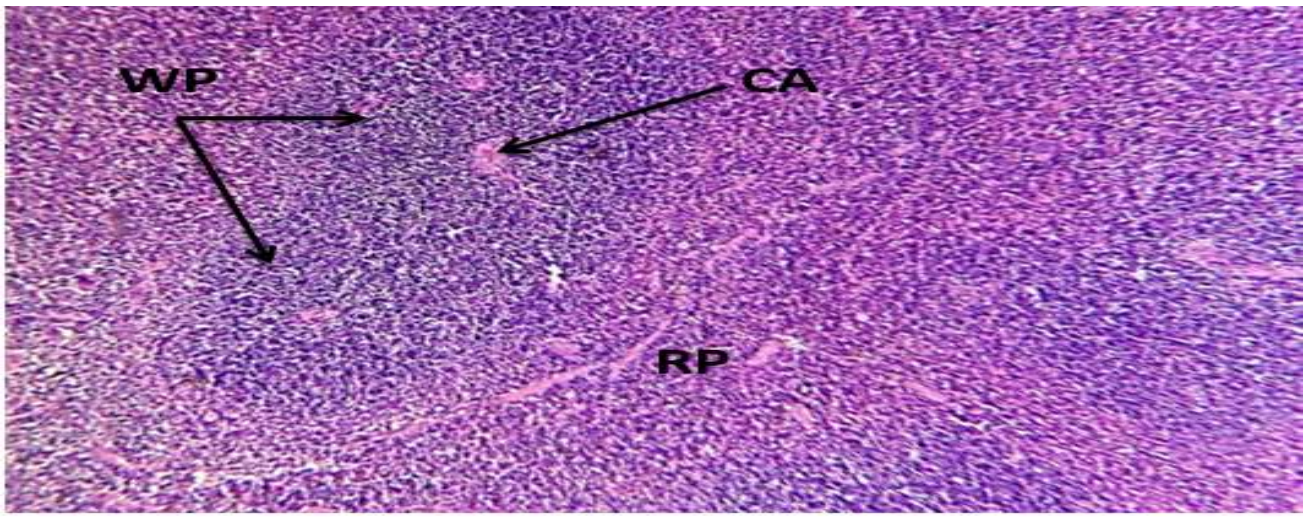

Figure 8: Splenic section of alcoholic extract group shows a normal tissue for white pulp (WP), red pulp (RP) and central artery (CA), H and E 100x

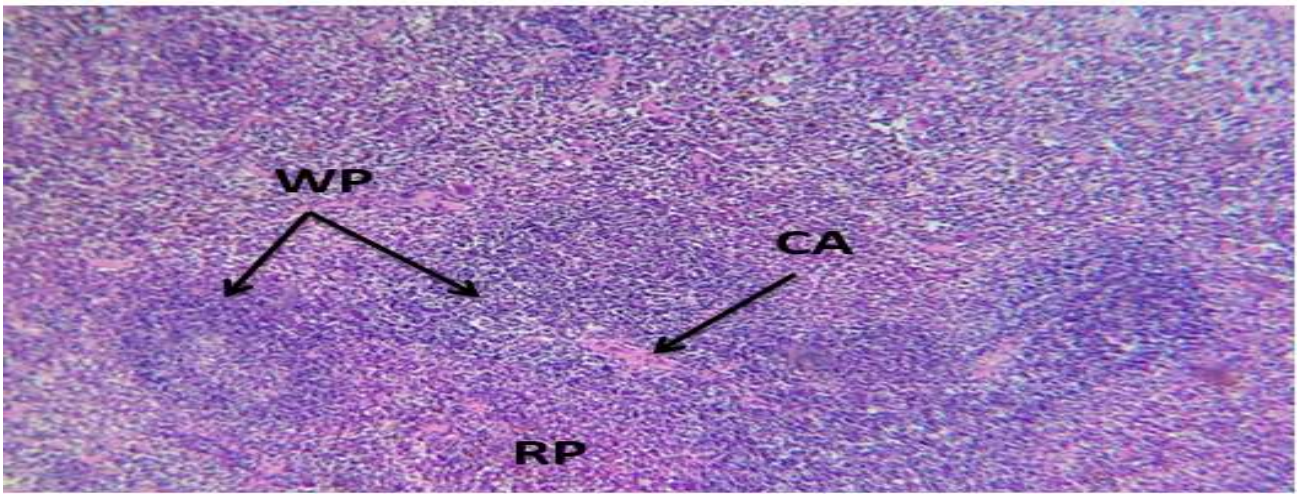

Figure 9: Splenic section of aqueous extract group shows a normal tissue for white pulp (WP), red pulp (RP) and central artery (CA), H and E 100x

About positive control groups, edema and necrotic materials appeared in the third (Figure 10) and fourth (Figure 11) groups as comparison with negative control group that showed normal splenic tissue (Figure 12). These histosplenic changes may attributed to host immune response against hydatid cyst and existence of different cytokines including interleukin and tumor necrosis factor (TNF), these cytokines lead to blood vessels expanding and somatic fluids accumulation in the peripheral tissue that create edema [51, 52]. While, necrosis can be happens due to the inflammatory reaction between defense cells and outer layer of hydatid cyst [53].

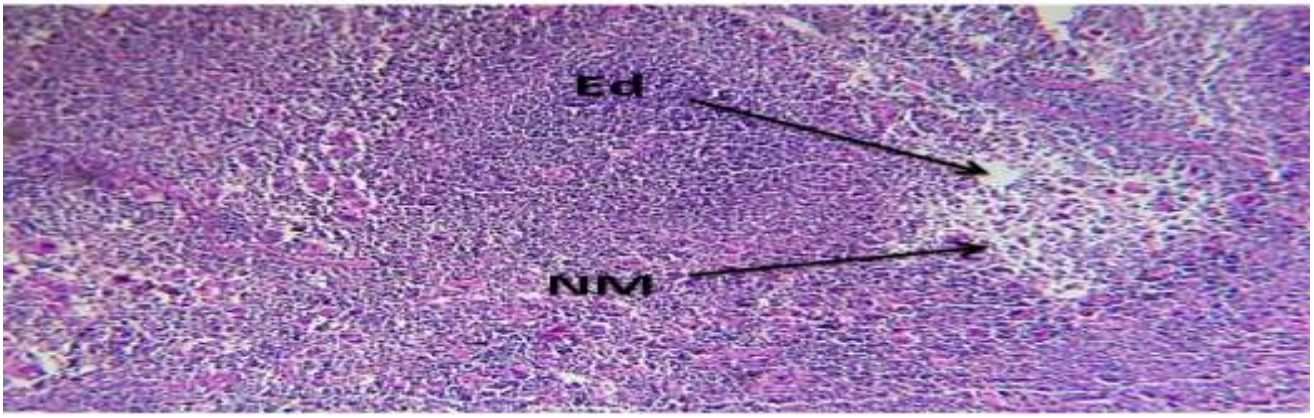

Figure 10: Splenic section of third positive control group shows edema (Ed) and necrotic materials (NM), H and E 100x

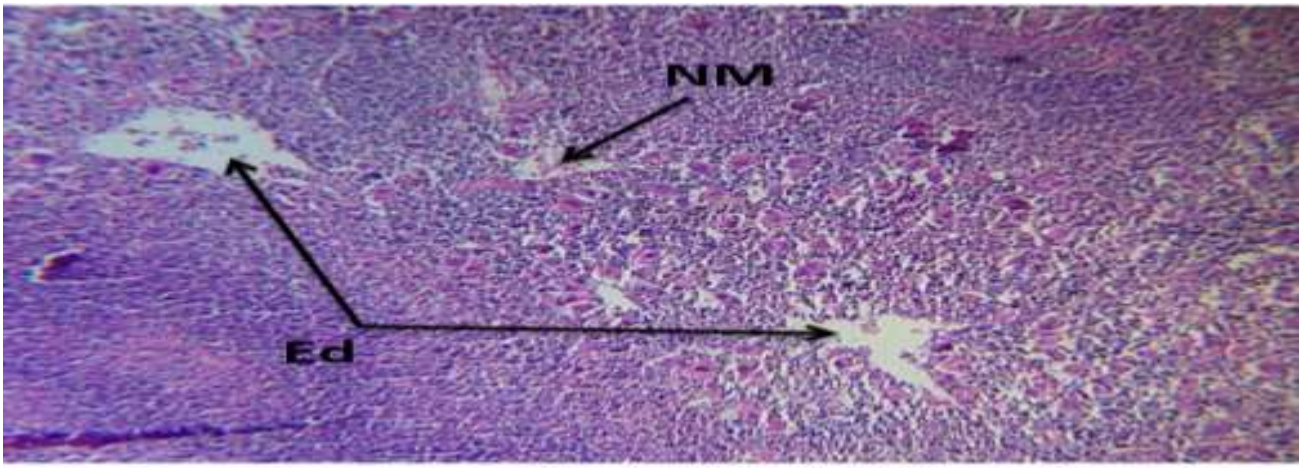

Figure 11: Splenic section of fourth positive control group shows edema (Ed) and necrotic materials (NM), H and E 100x

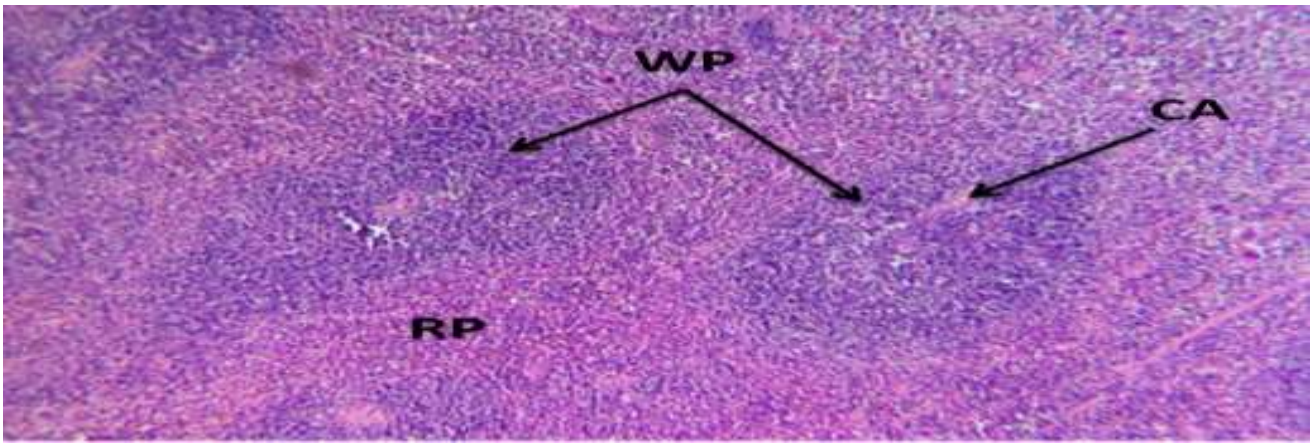

Figure 12: Splenic section of negative control group shows white pulp (WP), red pulp (RP) and central artery (CA) with normal sizes, H and E 100 xs

Conclusion

Hydatidosis has pathogenic effects such as rising of total cholesterol and triglyceride, decreasing of heavy density lipoprotein-C, in addition, histological effects for example degeneration, congestion of blood vessels, lymphocytes infiltration, edema and necrosis. These pathogenic effects increase with time progressing. Therefore, *C. cyminum* extracts are useful for attenuating of hydatidosis pathogenic effects because *C. cyminum* has some of active compounds. According to the present study, *C. cyminum* extracts are better than Albendazole due to no side effects are reported for *C. cyminum* extracts in this study.

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