# Effect Of Toxin Fractions Isolated From Protoscoleces And Hydatid Cyst Fluid Of Sheep Origin On Blood Picture In Mice

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## Abstract
Effect of toxin fractions of protoscoleces (P) and hydatid cyst fluid (HCF), of sheep origin on total and differential count of leucocytes was investigated. Mice of the species *Mus musculus* were injected with these TFs, at different concentrations(2.5&5µg), prior to infection with secondary hydatid disease. Results show that leucocytes are affected by TFs at both concentrations of both P and HCF as a decrease in TLC was noted when TFs of HCF & P at 3 and 15 days post infection but TLC increased at 30 days post infection compared with +ve control.
Key words: (toxin fraction, hydatid, blood picture, mice)

## Introduction
Hyatid disease is a cyclozoonotic disease of global importance. It is distributed all over the world, especially sheep and cattle rearing countries, including middle east [1].

Many trials have been carried out to cure the disease, using chemotherapeutic agents, but only partial success has been gained and, until now, it seems that surgery is still the treatment of choice, although it is difficult to apply in certain cases [2].

Recently, authors started to substitute the use of chemotherapeutic, agents by modulating the immune response in favor of the host using extracts, or fractions, obtained from plants, bacteria, and fungi [3, 4, 5] either to improve the immune response of the host or to act as protoscolecidals, and no definite results have been obtained, yet.

However, survival of established hydatid cysts in the host has been suggested to be associated with a particular immunomodulation activity on behalf of the parasite, which could be related to cytotoxicity of the cysts. The cytotoxicity of HCF on host immune cells has been demonstrated *in vitro* and *in vivo*, and the TFs in the fluid have been shown to be originated from the germinal layer [6, 7,8].

The present work in carried out with the main objective of investigating the effect of TFs, isolated from HCF and P of hydatid cysts of sheep origin, as possible immunomodulators in mice prior to infection with secondary hydatid disease**.**

## Materials And Methods
### Source of hydatid cysts
Hydatid cysts of sheep origin were obtained from infected livers of slaughtered sheep in Nineveh slaughter house.

### Isolation of cyst fluid
Protoscoleces were collected from the cysts, aseptically, according to Smyth [9]. Viability was estimated according to Smyth and Barrett [10] and only cysts with viability rate more than 90% were used. After centrifugation at 7600g (10000 rpm), using a cryofuge 6-4 (heraeus) for 10 minutes at 4°C, the supernatant (HCF) was separated and kept in sterile containers in a refrigerator at – 20°C until used.

### Separation of protoscoleces and cyst fluid toxin fractions
Cysts fluid fractions (CFFs) were separated according to Janssen *et al*. [11]. Ammonium sulphate was added to the cyst fluid and supernatant of homogenated protoscoleces* each one alone (49.35 gm/100ml) and the fluid was left in the refrigerator at 4°C for 24 hrs to give enough time for precipitation of protein (overnight) in refrigerator. The fluid was centrifuged.

An equal volume of chloroform was added to the supernatant. Two layers were formed after centrifugation. The chloroform layer was separated and half volume of methanol (chloroform: methanol = 2: 1, v/v) was added and centrifuged under the same conditions mentioned above. The supernatant was dried by rotary evaporator. The chloroform-methanol soluble fractions (CMSFs), or TFs, were kept in refrigerator at -20°C until use. At use, they were dissolved in few drops of chloroform and completed by phosphate buffer saline (PBS).

## Experimental design
30 Parasite-free, laboratory-bred, 5-6 weeks old male, BALB/c mice were used in the present study. They were injected, intraperitonealy (i.p.), with protoscoleces and hydatid fluid toxin fractions isolated from hydatid cysts of sheep origin as follows:

**Experiment 1**
5 mice were not treated with TFs and not infected with protoscoleces (-ve control group).

**Experiment 2**
5 mice were injected with approximately 2000 protoscoleces only, but not with TFs (+ve control group).

**Experiments 3-6**
In each experiment, 5 mice were injected with at the concentrations 2.5 and 5 µg of protoscoleces origin (experiment 3 and 4, respectively) and the same concentrations of (HCF) (experiments 5 and 6 respectively).

## Blood picture
mice anesthetized with diethyl ether and blood was obtained from the eye according to waynforth [12] at 3, 15,30 days post infection for experiment for on total and differential leukocyte count (TLC and DLC respectively). They were counted according to Dacie and lewis [13] counted. Control group mice were infected with protoscoleces only.

## Statistical analysis
Complete Randomized Design (CRD) and Duncan's Multiple Range Test were used to establish the difference between the means at the level $p \leq 0.05$.

## Results
Tables (1&2) show total and differential Leukocyte count in mice treated with TFs, isolated from HCF and P, respectively, at the concentrations 2.5 and 5 µg before infection with secondary hydatid cysts. Table (3) shows a comparison of TLC and DLC between mice injected

with TFs isolated from HCF and those infected with TFs isolated from protoscoleces at both concentrations .

It is obvious from table (1) that there is a significant increase in TLC in +ve C group compared with the -ve C one from the 3rd day post-infection. In mice treated with TFs isolated from HCF before infection, an increase in TLC was noticed at concentrations 2.5 & 5 µg compared with the -ve C from the 3rd day post-infection, but when these rates were compared with those of +ve C group, a decrease in TLC was noticed (at both concentrations) except at 30 days post infection when the concentration 5 µg was used where the opposite was noticed.

When TFs isolated from P were injected in mice before infections, alight increase in TLC in the first 3 days post-infection only, at both concentrations (2575&3550 at 2.5µg & 5µg respectively), was noticed compared with +ve C group, but started to decrease at day 15 post-infection until day 30 post-infection (table 2). When TLC in mice injected with TFs of HCF was compared with that of mice injected with TFs of P, it was noticed that TLC in mice injected with TFs of P was higher (2575) than in mice injected with TFs of HCF atboth concentrations until day 15 post-infection, where as the opposite was noticed at day 30 post-infection, at both concentrations (table 3).

For DLC, it is obvious from table (1) that neutrophils in +ve C group (41,45&44)where higher in number than those in –ve C group(35) from the 3rd day post-infection whereas the opposite was noticed in lymphocytes, but when TFs of HCF or P, at both concentrations where used, the opposite was obtained from the 3rd day post-infection. When TFs of HCF, at both concentrations, were used, neutrophils were higher than those of P.

However, the opposite was noticed with the lymphocytes, at both concentrations. For monocytes no difference was seen between -ve C and +ve C groups, at the first 3 days post-infections, but an increase was noticed at 15 days post -infections in +ve C group (Table 2).

It is obvious from table (3) that monocytes were lower in number when TFs of P at 2.5µg were used, at day 15 only post-infection at the concentration same it was lower (1.3). It was the other way around at 30 days post-infection, (4.5) when TFs of HCF were used at the concentration 5µg.

**Table (1) total and differential leukocyte count in mice treated with TFs isolated from HCF prior infection. N: Neutrophil, M: Monocyte, L: Lymphocyte**

| **Conc.** | **Mean ± SD** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Day** | **2.5 µg** | | | **5 µ g** | | | **Control +ve** | | | **Control** |
| **Parameter** | **3** | **15** | **30** | **3** | **15** | **30** | **3** | **15** | **30** | **-ve** |
| WBC | 1150 ± 70.7 a | 2100 ± 70.7 abc | 4900 ± 66.83 bcd | 3150 ± 1060.7 a-d | 1600 ± 28.3 ab | 5975 ± 3694.5 d | 2100 ± 458.3 abc | 5733.3 ± 1097 d | 5000 ± 866 cd | 900 ± 11.8 a |
| N | 35 ± 7.07 bc | 36 ± 5.66 bc | 18 ± 9.09 a | 33 ± 12.73 bc | 33.5 ± 6.36 bc | 25 ± 7.39 ab | 41 ± 4 c | 45.0 ± 4.0 c | 44.0 ± 4 c | 35 ± 3 bc |
| L | 64.5 ± 6.36 bc | 61.5 ± 2.12 bc | 82 ± 9.09 d | 62 ± 9.9 bc | 62 ± 8.49 bc | 74.25 ± 6.85 cd | 61 ± 6 bc | 52.0 ± 5.0 ab | 45.0 ± 4.0 a | 61 ± 5 bc |
| M | 0.5 ± 0.71 a | 2.5 ± 3.54 ab | 0 ± 0 a | 6 ± 1.41 ab | 4.5 ± 2.12 ab | 3.25 ± 6.5 ab | 2 ± 1 ab | 3.0 ± 1.0 ab | 8 ± 1 b | 2 ± 1 ab |

**Table (2) total and differential leukocyte count in mice treated with TFs isolated from P prior infection. N: Neutrophil, M: Monocyte, L: Lymphocyte**

| **Conc.** | **Mean ± SD** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Day** | **2.5 µ g** | | | **5 µ g** | | | **Control +ve** | | | **Control** |
| **Parameter** | **3** | **15** | **30** | **3** | **15** | **30** | **3** | **15** | **30** | **-ve** |
| WBC | 2575 ± 1150 abc | 4275 ± 478.7 bcd | 3825 ± 1851.8 a-d | 3550 ± 919.2 abc | 3700 ± 1254.3 abc | 3100 ± 1058.3 abc | 2100 ± 458.3 ab | 5733.3 ± 1097 d | 5000 ± 866 cd | 900 ± 11.8 a |
| N | 25.5 ± 4 bc | 19.5 ± 8.1 b | 20.5 ± 15.9 b | 6.5 ± 2.1 a | 24 ± 4.9 bc | 21.8 ± 5.1 b | 41 ± 4 d | 45.0 ± 4.0 d | 44.0 ± 4 d | 35 ± 3 cd |
| L | 73.3 ± 4.7 cd | 78 ± 9.9 de | 76.8 ± 16.6 de | 89 ± 1.4 e | 75.8 ± 5.1 de | 76 ± 5.4 de | 61 ± 6 bc | 52.0 ± 5.0 ab | 45.0 ± 4.0 a | 61 ± 5 bc |
| M | 1.5 ± 1.3 a | 1.3 ± 1.5 a | 2.8 ± 1.5 ab | 4.5 ± 0.7 b | 0.3 ± 0.5 a | 4.5 ± 3.4 b | 2 ± 1 ab | 3.0 ± 1.0 ab | 8 ± 1 c | 2 ± 1 ab |

**Table (3) comparison of total and differential count of leukocytes between mice treated with TFs of HCF and those of P, at different concentrations.**

| Dose | Period (day) | WBC | | N | | L | | M | |
|---|---|---|---|---|---|---|---|---|---|
| | | HCF | P | HCF | P | HCF | P | HCF | P |
| 2.5 | 3 | 1150 | 2575 | 35 | 25.5 | 64.5 | 73.3 | .5، | 1.5 |
| | 15 | 2100 | 4275** | 36 | 19.5 | 61.5 | 78.0 | 2.5 | 1.3 |
| | 30 | 4900 | 3825 | 18 | 20.5 | 82 | 76.8 | 0.0 | 2.8** |
| 5 | 3 | 3150 | 3550 | 33 | 6.5 | 62 | 89.0 | 6.0 | 4.5 |
| | 15 | 1600 | 3700 | 33.5 | 24.0 | 62 | 75.8 | 4.5 | 0.3* |
| | 30 | 5975 | 3100 | 25 | 21.8 | 74.25 | 76.0 | 3.25 | 4.5 |

Significant difference * at $p \leq 0.05$, ** at $p \leq 0.01$

## Discussion

The present study concentrated on the toxic effect of hydatid cysts on the host through study of the ability of toxin fractions, isolated from P and HCF of hydatid cysts, to immunmodulate immune response of the host against secondary hydatidosis.

One explanation of the survival and resistance of hydatid cysts in the body of the host is immunomodulation which occurs in the host on behalf of the parasit [14]. It is well documented that *E. granulosus* intermediate host, including humans, can often display specific cellular and humoral immune response [15, 16]. However, this does not obscure the lack of functional immunity against the primary hydatids [14].

Elevation of TLC in +ve C mice, compared with –ve C ones is expected and well known result, as continuation of existence of cysts in body will stimulate proliferation of immune cells [17]. These authors indicated that antigens 5 and B in HCF stimulated proliferation of T-cells in peripheral blood of patients infected with hydatid disease. In the present study, a difference in TLC of mice treated with TFs of P origin, compared with +ve C group , especially at the concentration 2.5µg, in the first 3 days postinfection was observed. This is in accordance with the demonstration presented by Janssen *et al*. [1] that, TFs at lower concentrations may have a higher effect. A possible explanation for this could be refered to the difference between TFs of P and those of HCF. It has been mentioned by Osuna *et al*. [18] that TFs of HCF are metabolites of the parasite whereas those of P are not metabolized. In this respect, however, previous authors [19] have demonstrated that metabolism may play a role in changing the toxicity of some fractions. Difference between the toxicity of TFs of P origin and those of HCF origin on neutrophils, lymphocytes and moncytes (DLC) may support this speculation. There is also the possibility that TFs of HCF may come from degeneration of immature P, leading to existence of different precursors of different nature, compared with those in mature P. However, this needs further investigation, in detail.

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*protoscoleces were suspended with phosphate buffer saline (1:4 v/v), then freezed and thawed two times prior homogenization with ultra sonicator at 24 waves for 3-6 minutes, finally the liquid was centrifuged at 14000rpm for 15minutes to separate the supernatant (crude extract of P).

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# تأثير الأجزاء السامة المعزولة من الرؤيسات الأولية والسائل العدري من أصل أغنام على صورة الدم في الفئران

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**الملخص**

درس تأثير الأجزاء السامة للرؤيسات الأولية والسائل العدري المعزولة من الأكياس العدريه من أصل أغنام على خلايا الدم البيضاء. حقنت فئران من نوع *Mus musculus* بهذه الأجزاء السامة ،عند التراكيز مختلفة (2.5,5µg)، قبل إحداث الاصابه بمرض الأكياس العدريه الثانوي. أظهرت النتائج أن خلايا الدم البيضاء تأثرت بالأجزاء السامة في التراكيز المختلفة للرؤيسات والسائل العدري، اذ لوحظ انخفاض التعداد الكلي لخلايا الدم البيض عند استخدام الأجزاء السامة لسائل الكيس العدري بالتركيزين بعد ٣،١٥ يوما من إحداث الاصابه إلا إن العدد الكلي ارتفع بعد ٣٠ يوما من إحداث الإصابة مقارنة بضابط التجربة الموجب.