The use of some natural Dyes for detection of the viability of *Echinococcous granulosus* protoscolices in vivo

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Abstract

An aqueous extract for natural dyes which extracted from two plants Shamender and Kujarat. These two dyes were used for detection of viability of protoscolices in hydatid cyst of Echinococcus granulosus as compared with traditional dye (Eosin).

These natural dye which extracted from Shamender stained both protoscolices and hydatid sand more better than which extracted from Kujarat. Both extracts were an important dyes and successfully used for staining and diagnosis a protoscolices of hydatid cysts

Introduction

Echinococcosis considered a Zoonoses disease between human and carnivores animals (as intermediate hosts) and dogs and most canine animals (as final hosts) (Carmena *et., al.* 2005; Slifko *et., al.* 2000; Oku *et., al.* 2004).
The cestoda worm Echinococcus granulosus the main cause of echinococcosis disease which lived in small intestine as adult worms in dogs (Oku *et., al.* 2004; Benito *et., al.* 2006). The ova were released with infected stray dog's feces in soil, water, grass and plants, so, at this way can infestation spread and transmitted the infection through the hosts. When these ova passé through any method to the human or to the carnivores animals, these ova can hatch and growth to the larval stage which called hydatid cyst (Al- fatlawi, 2004). At this state all organs and tissues of an infected body were damaged and a high percentage of infestation at liver and lungs (Heath,1971).
The hydatid cyst consist of protoscolices with sand fluid surrounded with layers (germinal layer) (Eckert *et., al.* 2001). All the laboratory study about the Hydatidosis focused on laboratory infection, protoscolices growth and extraction (Al- fatlawi, 2004), antigens isolation from protoscolices and fluid (Carmena *et., al.* 2005; Oku *et., al.* 2004). Or using some plants extracts and study the effects on hydatid cysts activity and variability (Al- kennany & Ali 2004; Dirican *et., al.* 2008). So, all researches depended on the activity and variability of protoscolices inside hydatid cyst.
The plant Beta vulgaris L. one of the most important which was from the family chenopoiacees, it was a huge rooting plant and from an ancient the human used as food, because of contain a high bioenergy especially sucrose, amino acids. Vitamins C, B2, B1, PP and A, also, with many necessary mineral salts like: brome, manganese, lithium, rubidium and strontium, furthermore, many studies founded that this plant used as a drug for intestinal inflammation, bile duct disease and antimicrobial (kubassy, 2004).

The Karkadea (as it name in Egypt and some countries) or kujarat (as it name in Iraq, Syria and Arabian gulf) *Hibiscus sadariffa L* one of plant under the family Malvacea and almost plants herbs and seasonal, with green- red stem, dark red leaves and flowers and 1.5 meter as high (Egypt pub., 1980). There are many studies about this plant were done, like the main structures of chemicals component of pigments, which founded that mostly phlaphonoides structures Delphinidin- 3- Sambubioside and Cyanidin- 3- Sambubioside (Morton, 1987; Muller & Franz, 1992; Tseng *et., al.* 1997).

In Iraq one of the most governorate (Al-qadisiyah) famous with culturing Al-Kujarat in their lands, and used this plant as a favor in yogurts and diary products. Also, a medical important for this plant to treated many diseases like: athero sclerosis, activity of peristaltic movement, tranquilizer, treatment of magrinia, hypotensive, arthritis and antiacidity because of a high amount of plant acids and vitamin C (Al-diwania news, 2009).

The aim of this study was to focused viewed about these plants and the relationship with our life even than as eaten products, also, to detect if the pigments inside these plants can be useful for laboratory diagnosis in biological studies.

Material and Methods

1- the method of Smyth & Barret (1980) were used for prepared eosin dyes 0.1%.

2- for prepared a shaminder and kujarat dyes: a total of 50 gram of shaminder fruit and 10 gram of dry kujarat flowers each one alone were washed and cutted, then, boiling in 100 ml. distilled water, the results were filtered and put in a clean sterilized dry glass vials with 0.25 gram of sodium azide (for fixed molds and fungi growth). The solution were put in cooled room at 14C0, from time to other the vials were shacked.

3- Samples collection: A weekly visiting to the Al-Basrah abattoir for detection and collection hydatid cysts. Each cyst were isolated in a clean container then bring it to the laboratory for study and a procedure of pigment were done. A procedure of (Smith & Davies 1974) were used for isolation the liquid and protoscolices.

The sediment protoscolices from hydatid cyst were transfer to a clean Petri dishes in three groups (with 10- 15) each one with one ml. of; the first one put eosin stain, the second with Kujarat dye and the last with shaminder dye. Each were replicate between 10-15 time.

Each plate were moved as circular and left for 15 minutes, then, examined under dissected microscope. Later, recognized the dead and lived and pictured by camera digital.

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Results

1- Eosin dye:

The result Showed a different in color between live and dead protoscolices, a live one with green, while, the dead with red color. But, the hydatid liquid without any pigment. The dye show a fixative for 24 hour, but later the colors changed, as shown in pictures (1, 2, 3, 4).

2- Kujarat dye:

A different colors were founded in this dye, the live protoscolices with brownish, while, the dead with pink. Furthermore, the hydatid liquid pigment with pink but without any diagnosis for each particles inside it. The dye was fixed for ten days, pictures (5, 6, 7, 8, 9, 10).

3- Shaminder dye:

A protoscolices with reddish cooler with shaminder dye as shown in picture (11), while, the dead protoscolices without any pigment (picture, 12, 13). Also, the hydatid liquid with clear pigment (picture, 14, 15, 16). The dye fixed for week (picture, 17, 18).

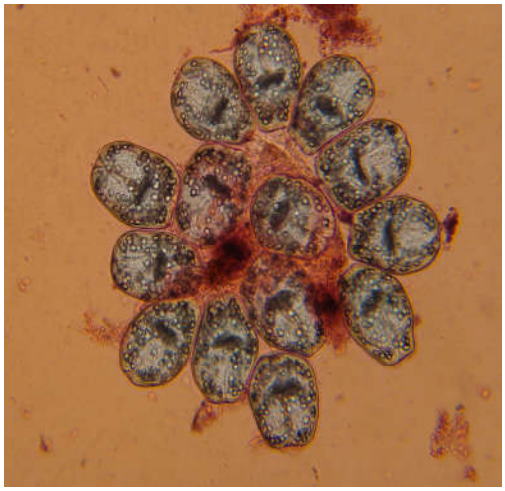

fig (1): Protoscolices of hydatid cyst stained with eosin dye X10

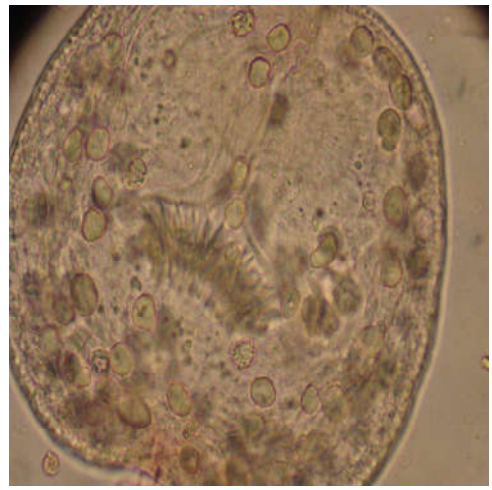

fig (٢): Protoscolices of hydatid cyst stained with eosin dye X40

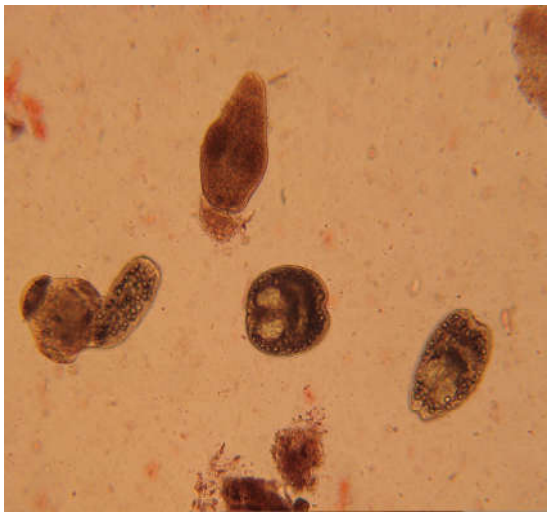

fig (3): protoscolices stained with Kujarat dye. 10X.

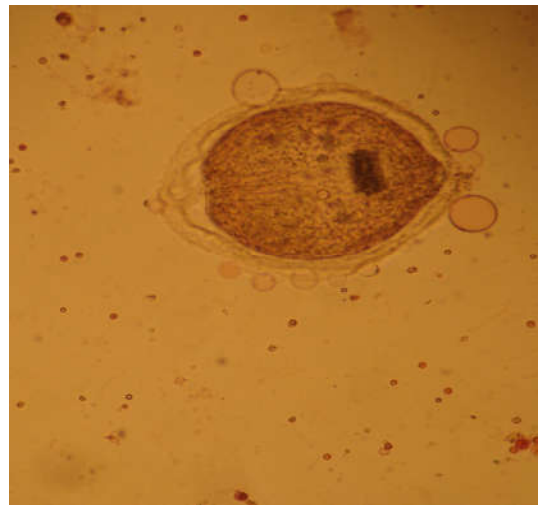

fig (4): One of protoscolices stained with Kujarat dye. 40X

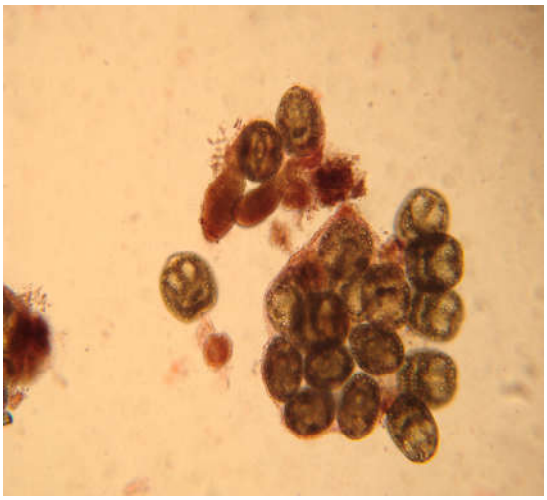

fig (5): Protoscolices stained with Kujarat dye. 10X.

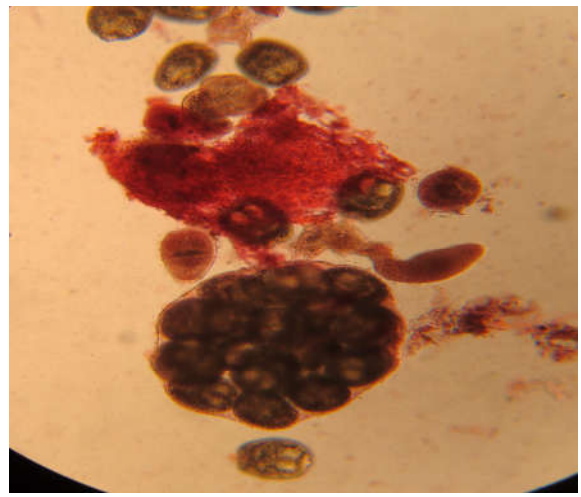

fig (6): Protoscolices stained with shaminder dye. 20X

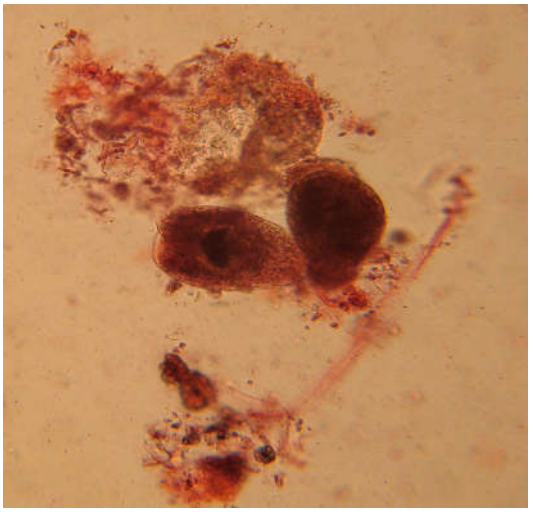

fig (7): Protoscolices stained with Shaminder stain. 20X

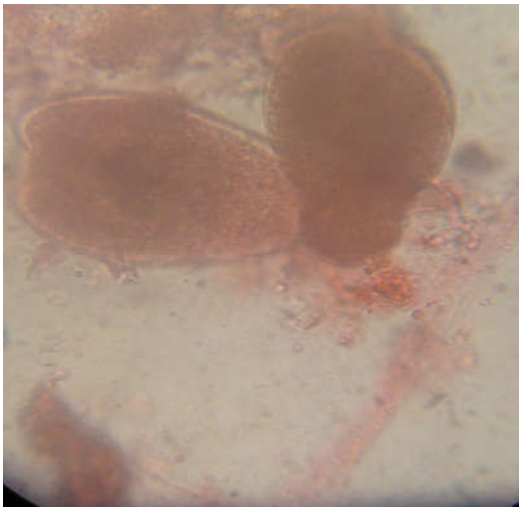

fig (8): Protoscolices stained with Shaminder stain. 40X

Discussion

The hydatid disease one of the most important parasitic hyperendemic which distribution all over the world, and Iraq one of them, this is because a high number of stray dogs all over the country with a high infestation inside body (El-Smeary *et., al.* 2005).

The epidemiological studies all over the country showed an increase in number of dogs infected with *E. granulosus*, in some cases the percentage infection reached to the 80% (Abdul wadood, 2003).

The current study showed that some natural dye which extract from some plants can pigmented the protoscolices of hydatid cyst of *E. granulosus* and can be a distinguish dye between live and dead one, furthermore, these dyes can in some pigment the hydatid liquid with all particles inside it. With a fixed dye for along time, and this prove make that not only a traditional dye make a distinguish and used to recognize the mortality of protoscolices.

This result agreed with Al-Idreesy (2005) which focused that Kujarat dye can used a pigment for staining some cestodes and nematodes worms and fixed for along time as compared with traditional dye (acetocarmine).

The new in this study was used shaminder dye for laboratory study and can used this pigment for any laboratory purposes when it use moreover as a food material.

So, it can do a further studies about this two natural dyes and detection a chemical components and used for more laboratory purposes because of the facilities and available in any time and can used it more than other dyes.

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الملخص العربى

استخدام بعض الأصباغ الطبيعية في تقدير حيوية الرووَيسات الأولية لطفيلي لمشوكات العدرية معمليا

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تم في هذه الدراسة إجراء استخلاص مائي للحصول على صبغات طبيعية من نباتي الكجرات (الكركدية) والشمندر، وذلك لغرض استخدامهم في الكشف عن حيوية الرؤويسات الأولية للأكياس المائية ومقارنتها مع الصبغة التقليدية الشائعة الاستخدام (الايوسين المائية).
لوحظ ثبات الصبغتين أعلاه أكثر من الصبغة التقليدية ولفترة وصلت الى ١٠ أيام، كما لوحظ إن الصبغة لم تقتصر على الرؤويسات كما هو الحال في الايوسين بل تعدتها الى صبغ السائل العدري وتمييز محتوياته.
عليه أظهرت هذين النباتين أهمية الصبغات التي تحويها، كما محتوياته وخصوصا عند استخدام صبغة الشمندر.أظهرت نجاح في صبغ وتمييز الرؤويسات الأولية للمشوكات الحبيبية.