

DETERMINATION OF LIPIDS AND GLUCOSE CONTENT IN HYDATID CYSTS OF ECHINOCOCCUS GRANULOSUS ISOLATED FROM DIFFERENT INTERMEDIATE HOSTS (SHEEP, GOATS, CATTLE AND HUMAN) TISSUES

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(Accepted for publication: June 9, 2013)

Abstract:

This study included comparative biochemical composition of hydatid fluid, protoscolices, infected and non-infected tissues isolated from liver and lungs of infected sheep, goats, and cattle slaughtered in Duhok abattoirs during the period from Nov. 2009 to Apr. 2010. Also hydatid fluid of cysts surgically removed from humans in Azadi Teaching Hospital, Duhok during the period from Mar. 2010 to Jul. 2010. Protoscolices contained higher levels of Lipids, triglycerides and cholesterol with the highest being in cattle liver (63.81 ± 1.434 mg/100g, 38.28 ± 1.277 mgs/100gs and 28.85 ± 0.795 mgs/100gs, respectively) and infected cattle liver and lung tissues (57.78 ± 1.3 mgs/100gs and 51.91 ± 1.299 mgs/100gs, respectively). Hydatid fluid contained high levels of glucose with the highest in hydatid fluid of sheep cysts (Liver: 37.41 ± 0.384 mgs/dl and Lung: 38.98 ± 0.424 mgs/dl) and infected sheep Lung tissues (48.12 ± 0.475 mg/100g).

KEYWORDS: *Echinococcosis, Echinococcus granulosus, HCS chemical composition, Lipid, Cholestrole, Glucose.*

INTRODUCTION

Hydatidosis or Echinococcosis is an endemic zoonotic parasitic disease, which is common between human and animals and has a worldwide spread. *Echinococcosis* has been paid more attention because of its medical and veterinary importance. In the developing countries, the people who live in villages are more prone to infection due to increasing the chances of contact with domestic animals particularly dogs and wild animals belonging to this family (Craig *et al.*, 1992; Craig *et al.*, 1996; Dalian and Mobedi, 1997 and Biava *et al.*, 2001).

Different species and sub-species (strains) of *Echinococcus* have been reported from most areas where infection is endemic; most of these reports give valuable information about the structure and natural specification of the parasite (Bowles & McManus, 1992; Bowles & McManus, 1993). This variation definitely affects the epidemiology, pathology, prophylaxis and control of this disease (Bowles & McManus, 1993; Bowles & McManus, 1994).

In addition, there are some evidences indicating that some strains of the parasite are more infectious to man than others (Thompson, 1995). Therefore, the study of this parasite assigns a significant position in research particularly, when there are several intermediate hosts and different transferable ways of infection

to human in these regions (Eslamirad *et al.*, 2000). Biochemical studies on the hydatid cyst components has important role in determining the sub-species taxonomy of this parasite (Thompson *et al.*, 1994).

Regarding Iraq, hydatidosis is endemic in human, and some domestic animals including sheep, cattle, goats and camel (act as intermediate hosts), while dog and wolf act as final hosts (Al- Nakeeb, 2004). Despite to the economical and medical importance of hydatidosis, little attention has been paid to the chemical composition and comparative metabolic studies of the parasite and its host. MacPherson *et al.*, (1985), proposed a strategy for the chemotherapy of infectious diseases utilizing biochemical differences, and stated that the inhibition of enzyme systems that are crucial to the parasites but not the host may be the basis of rational approach to chemotherapy of the parasite. However, biochemical studies are also useful in differentiating strains of *E. granulosus* in different countries (Radfar and Iranyar, 2004). The strain characterization is particularly important in regions where more than one species of livestock intermediate host exists and where there is the possibility of different cycles of transmission and sources of infection for humans (Thompson *et al.*, 1995).

This study proposed to determine the chemical components of hydatid cysts (HCs)

fluid and protoscolices of cysts isolated from liver and lung tissues of various intermediate hosts, in addition to infected and noninfected host tissues.

MATERIALS AND METHODS

Materials:- The present study include biochemical study on 38 fertile hydatid cysts isolated from the liver and Lungs of infected sheep, goats and cows slaughtered at Duhok abattoir (Table 1) during the period between November 2009 to April 2010. In addition, 8 hydatid cyst fluids aspirated from humans during surgical removal of cysts from patients at Azadi Teaching Hospital in Duhok city during the period from March 2010 to July 2010. Samples were collected carefully and kept in cool box containing crushed ice and transported to Parasitology laboratory of College of Education, University of Zakho.

Table (1): The number of samples used in this study from different sources

Host	Liver	Lungs	Total
Sheep	5	5	10
Cattle	5	5	10
Goats	5	5	10
Human	4	4	8
<i>Grand Total</i>	19	19	38

Buffer solution: - Buffer solutions used in this study were prepared as described by Saeed and Al-Habbib, (1990). They included Tris-HCl buffer (pH 7.4) for determination of lipid profiles (total lipids, triglycerides and cholesterol), glucose.

Excision of the cyst from infected organs: The cyst was removed from the infected organ and transferred to a crystallizing basin, washed several times with physiological buffer solution (PBS) (pH 7.4) then opened and the cyst content was transferred to a clean container, then the sample was subjected to the following protocol.

Hydatid fluid: The hydatid fluid of each sample was centrifuged at 4000 rpm for 20 minutes, Millipore filtered; the supernatant stored in a deep freezer at -40°C until used.

Protoscolices: The protoscolices obtained from each fertile hydatid cysts were washed several times by repeated centrifugation with PBS (pH 7.4). The precipitate was mixed with the desired buffer (0.5 gm protoscolices with 5 ml buffer), homogenized using glass homogenizer connected to a variable speed

stirrer. The homogenizer was placed in a beaker containing crushed ice (Mero *et al.*, 1988). After complete homogenization for 5-10 minutes, the protoscolices extract was centrifuged at 4000 rpm for 20 minutes; the supernatant was collected and stored in a deep freezer at -40°C until used.

Infected and non-infected organ tissues: The liver and lung tissues of infected animals whose cyst were selected, in addition, tissues of non-infected animals (Sheep, Goats and Cattle) were used as control. The tissues were washed several times with PBS (pH 7.4) and homogenized with a desired volume of the buffer (0.5 gm tissues with 5 ml of buffer) (Mero *et al.*, 1988). The samples were treated in the same way as previously described; the supernatant was collected and stored in deep freezer at -40°C until used.

Determination of total lipids of hydatid cyst fluid: The total lipids content of hydatid cyst fluid was determined using total lipids Kit (E. Merck, Kit. No. 3321).

Determination of total lipids of protoscolices and tissues: The total lipids content of protoscolices and the tissues were determined by Soxhlet extraction method (Tangprawat, 2006):

- 1- Samples of the infected tissue (5 gm) and noninfected tissue (5 gm) and 1 gm of protoscolices were weighted separately.
- 2- The samples were dried in oven at 50 °C for 48 hours.
- 3- The dried tissue was grinded using electric grinder to a fine powder.
- 4- The powder of each sample was weighted (first weight) and placed in a small thimble.
- 5- The sample in a dried extraction thimble was covered with cotton plug.
- 6- One hundred fifty -180 ml of petroleum ether (B.P) was boiled in bottled flask (250 ml).
- 7- The total lipid was extracted by petroleum ether for 4 hours at condensation rate of 5-6 drops per second by heating solvent in boiling flask.
- 8- The solvent separated from the extracted fat by putting the boiling flask by evaporating the excess solvent.
- 9- The boiling flask with extracted fat was dried in a hot air oven at 100 °C for 30 min, then cooled and weighted.

The total lipid content was calculated by using the following formula; the results were expressed as mg/100 g for protoscolices and tissues.

$$\text{TotalLipids\%} = \frac{(\text{Weight of flask and fat} - \text{Weight of flask}) \times 100}{\text{Weight of dried sample}}$$

Total Lipid = % Total Lipid X Weight of dried sample

Determination of Triglycerides: Triglycerides content of the samples was determined using triglycerides Kit (Biolabo Reagents- France). The Cholesterol content was determined using cholesterol kit (Biolabo Reagents- France). Glucose analysis of hydatid cyst fluid, protoscolices, infected and non-infected tissues: The Glucose content was determined using glucose Kit (Biolabo Reagents- France).

Statistical analysis: One way analysis of variance (ANOVA) was used for statistical analysis and for comparison between the results (Cohen, 2003).

RESULTS

The contents of total lipids, triglycerides and cholesterol of hydatid cysts, infected and non-infected sheep liver and lung tissues are shown in Table (2) it is obvious from the results that total lipids, triglycerides and cholesterol contents of protoscolices of liver and lung cysts were higher than that of hydatid fluid.

Table (2): Lipids and glucose contents of hydatid cyst, infected and non-infected sheep liver and lung tissues (N: 5).

Parameters	Organ	Hydatid fluid (mg/dl)	Protosco-lice (mg/100g)	Infected tissue (mg/100g)	Non-infect-ed tissue (mg/100g)
Total lipids	Liver*	29.54 ± 0.664	53.17 ± 1.196	48.15 ± 1.083	58.19 ± 1.307
	Lung*	26.54 ± 0.664	47.77 ± 1.196	43.26 ± 1.083	52.28 ± 1.307
Triglyce-ride	Liver	9.38 ± 0.313	31.9 ± 1.063	25.33 ± 0.845	33.78 ± 1.126
	Lung	9.526 ± 0.212	32.39 ± 0.721	25.73 ± 0.571	34.3 ± 0.762
Cholest-erol	Liver	7.578 ± 0.209	24.24 ± 0.669	20.46 ± 0.566	26.51 ± 0.733
	Lung	7.396 ± 0.18	23.67 ± 0.576	19.97 ± 0.488	25.88 ± 0.632
Glucose	Liver*	37.41 ± 0.384	26.21 ± 0.326	46.36 ± 0.43	62.77 ± 0.513
	Lung*	38.98 ± 0.424	27.55 ± 0.36	48.12 ± 0.475	64.88 ± 0.567

Also the total lipids, triglycerides and cholesterol contents of non-infected liver tissues were higher than that of infected tissues. Generally the total lipids of hydatid cysts of liver along with infected and non-infected tissues were higher as compared with their correspondent lung cysts and tissues and these differences were statistically significant ($P \leq 0.05$).

Hydatid fluid of both liver and lung cysts showed the highest glucose contents, which were

37.41±0.384 and 38.98±0.424 mgs/dl, respectively. Furthermore, non-infected liver and lung tissues showed higher glucose contents as compared with infected (Table 2).

Table (3) shows the contents of total lipids, triglycerides and cholesterol of HCs infected and non-infected goats liver and lung tissues. The total lipids, triglycerides and cholesterol contents of protoscolices of liver and lung cysts were higher than that of hydatid fluid (Table 3).

Table (3): Lipids and glucose contents of hydatid cyst, infected and non-infected goats liver and lung tissues (N: 5).

Parameters	Organ	Hydatid fluid (mg/dl)	Protosco-lice (mg/100g)	Infected tissue (mg/100g)	Non-infect-ed tissue (mg/100g)
Total lipids	Liver*	34.56 ± 0.778	62.21 ± 1.399	56.34 ± 1.266	68.09 ± 1.531
	Lung*	31.05 ± 0.778	55.89 ± 1.398	50.61 ± 1.266	61.17 ± 1.53
Triglyce-ride	Liver	10.88 ± 0.363	37 ± 1.234	29.38 ± 0.981	39.18 ± 1.306
	Lung	11.05 ± 0.245	37.57 ± 0.837	29.84 ± 0.665	39.78 ± 0.886
Cholest-erol	Liver	8.714 ± 0.24	27.88 ± 0.77	23.52 ± 0.649	30.49 ± 0.842
	Lung	8.508 ± 0.208	27.21 ± 0.664	22.96 ± 0.56	29.77 ± 0.727
Glucose	Liver*	27.71 ± 0.333	17.97 ± 0.284	35.5 ± 0.372	49.78 ± 0.447

Lung*	29.07 ± 0.367	19.13 ± 0.314	37.03 ± 0.412	51.61 ± 0.493
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Also the total lipids triglycerides and cholesterol contents of non-infected liver tissues were higher than that of infected tissues. Generally the fat contents of hydatid cysts of liver along with infected and non-infected tissues were higher as compared with their correspondent lung cysts and tissues and these differences were statistically significant ($P \leq 0.05$).

Hydatid fluid of both liver and lung cysts showed the highest glucose contents which were

27.71±0.333 and 29.07±0.367 mgs/dl, respectively. Furthermore, non-infected liver and lung tissues showed higher glucose contents as compared with infected (Table 3).

Table 4 shows the contents of total lipids triglycerides and cholesterol of HCs, infected and non-infected cattle liver and lung tissues.

The total lipids, triglycerides and cholesterol contents of protoscolices of liver and lung cysts were higher than that of hydatid fluid (Table 4).

Table (4): Lipids and glucose contents of hydatid cyst, infected and non-infected cattle liver and lung tissues (N: 5).

Parameters	Organ	Hydatid fluid (mg/dl)	Protosco-lice (mg/100g)	Infected tissue (mg/100g)	Non-infect-ed tissue (mg/100g)
Total lipids	Liver*	35.45 ± 0.796	63.81 ± 1.434	57.78 ± 1.3	69.83 ± 1.57
	Lung*	31.85 ± 0.796	57.33 ± 1.434	51.91 ± 1.299	62.74 ± 1.57
Triglyce-ride	Liver	11.26 ± 0.375	38.28 ± 1.277	30.4 ± 1.013	40.53 ± 1.352
	Lung	11.43 ± 0.254	38.87 ± 0.865	30.87 ± 0.688	41.16 ± 0.917
Cholest-erol	Liver	9.016 ± 0.248	28.85 ± 0.795	24.34 ± 0.672	31.55 ± 0.872
	Lung	8.8 ± 0.214	28.16 ± 0.686	23.76 ± 0.58	30.8 ± 0.752
Glucose	Liver*	12.04 ± 0.253	4.652 ± 0.215	17.95 ± 0.282	28.78 ± 0.34
	Lung*	13.08 ± 0.28	5.534 ± 0.238	19.11 ± 0.313	30.17 ± 0.375

Also the total lipids triglycerides and cholesterol contents of non-infected liver tissues were higher than that of infected tissues.

Generally the fat contents of hydatid cysts of liver along with infected and non-infected tissues were higher as compared with their correspondent lung cysts and tissues and these differences were statistically significant ($P \leq 0.05$).

Hydatid fluid of both liver and lung cysts showed the highest glucose contents which were 12.04±0.253 and 13.08±0.28 mgs/dl, respectively. Furthermore, non-infected liver and lung tissues showed higher glucose contents as compared with infected (Table 4).

Table 5 shows the contents of total lipids triglycerides and cholesterol of hydatid fluid removed from liver and lungs of human. The total lipids of hydatid fluid of liver was 29.22±0.762 mg/dl which was higher than that in lungs which was 26.36±0.708 mgs/dl, the triglyceride was slightly higher in lungs and cholesterol was slightly higher in liver. But these differences were statistically non-significant ($P > 0.05$).

Hydatid fluid of lung cysts showed the highest glucose contents which was 16.02 ±

0.356 mgs/dl as compared with that of liver (14.88 ± 0.294 mg/dl) but this differences was statistically non-significant ($P > 0.05$) (Table 5).

Table (5): Lipids and glucose contents of hydatid fluid of cysts removed from human (mg/dl) (N: 4).

Parameters	Liver	Lung	Normal values
Total lipids*	29.22 ± 0.762	26.36 ± 0.708	----
Triglyceride	9.178 ± 0.394	9.445 ± 0.23	< 250
Cholesterol	7.328 ± 0.26	7.165 ± 0.227	< 200
Glucose*	14.88 ± 0.294	16.02 ± 0.356	50-75

Table (6) shows comparison between the biochemical compositions of hydatid cysts isolated from liver of various intermediate hosts. The levels of total lipids, triglycerides and cholesterol were higher in protoscolices of cysts isolated from sheep, goats and cattle with the highest being in cattle cysts.

The hydatid fluid of sheep liver cysts and human liver cysts approximately contained similar levels of total lipids, triglycerides and cholesterol (Table 7).

The glucose level of hydatid fluid was higher than that of protoscolices with the highest (37.41 \pm 0.384 mgs/dl) in hydatid fluid of sheep cysts.

Table (6): Comparison between biochemical compositions of hydatid cysts isolated from livers of different hosts.

Parameters	Sheep (N:5)		Goat (N:5)		Cattle (N:5)		Human (N:4)
	Hydatid fluid (mg/dl)	Protoscolices (mg/100g)	Hydatid Fluid (mg/dl)	Protoscolices (mg/100g)	Hydatid Fluid (mg/dl)	Protoscolices (mg/100g)	Hydatid Fluid (mg/dl)
Total lipids*	29.54 \pm 0.664	53.17 \pm 0.196	34.56 \pm 0.778	62.21 \pm 1.399	35.45 \pm 0.796	63.81 \pm 1.434	29.22 \pm 0.762
Triglyceride*	9.38 \pm 0.313	31.9 \pm 1.063	10.88 \pm 0.363	37 \pm 0.234	11.26 \pm 0.375	38.28 \pm 1.277	9.1775 \pm 0.394
Cholesterol*	7.578 \pm 0.209	24.24 \pm 0.669	8.714 \pm 0.24	27.88 \pm 0.77	9.016 \pm 0.248	28.85 \pm 0.795	7.3275 \pm 0.26
Glucose*	37.41 \pm 0.384	26.21 \pm 0.326	27.71 \pm 0.333	17.97 \pm 0.284	12.04 \pm 0.253	4.652 \pm 0.215	4.88 \pm 0.294

Protoscolices showed higher levels of total lipids, triglycerides and cholesterol, but these values were lower than those of liver cysts.

Glucose contents of hydatid fluid were higher than those of protoscolices in cysts isolated from

the studied animals, with the highest being in sheep cyst. Also both protoscolices and hydatid fluid of sheep lung cysts showed higher values of uric acid (Table 7).

Table (7): Comparison between biochemical compositions of hydatid cysts isolated from lungs of different hosts.

Parameters	Sheep (N:5)		Goat (N:5)		Cattle (N:5)		Human (N:4)
	Hydatid fluid (mg/dl)	Protoscolices (mg/100g)	Hydatid Fluid (mg/dl)	Protoscolices (mg/100g)	Hydatid Fluid (mg/dl)	Protoscolices (mg/100g)	Hydatid Fluid (mg/dl)
Total lipids*	26.54 \pm 0.664	47.77 \pm 1.196	31.05 \pm 0.778	55.89 \pm 1.398	31.85 \pm 0.796	57.33 \pm 1.434	26.36 \pm 0.708
Triglyceride*	9.526 \pm 0.212	32.39 \pm 0.721	11.05 \pm 0.245	37.57 \pm 0.837	11.43 \pm 0.254	38.87 \pm 0.865	9.445 \pm 0.23
Cholesterol*	7.396 \pm 0.18	23.67 \pm 0.576	8.508 \pm 0.208	27.21 \pm 0.664	8.8 \pm 0.214	28.16 \pm 0.686	7.165 \pm 0.227
Glucose*	38.98 \pm 0.424	27.55 \pm 0.36	29.07 \pm 0.367	19.13 \pm 0.314	13.08 \pm 0.28	5.534 \pm 0.238	16.02 \pm 0.356

DISCUSSION

In sheep, goats, and cattle, the highest levels of total lipid, triglycerides and cholesterol were present in protoscolices of the hydatid cysts from both lungs and liver as compared with their levels in hydatid cyst fluids. These results agree with those reported for sheep protoscolices and hydatid cyst fluids (Frayha and Haddad, 1980). The presence of cholesterol, triglycerides and phospholipids in hydatid cyst fluid may be due to rapid diffusing properties of these compounds through the hydatid cyst membranes (Bahr *et al.*, 1979).

The present study also showed that the lipid, triglycerides and cholesterol contents of goats

and cattle hydatid cyst protoscolices and fluids were significantly higher as compared with hydatid cyst of sheep and human. These results agree with those reported by McManus (1981) who found that the lipids content of goats hydatid cyst fluid was significantly higher than hydatid cyst fluid from sheep, but it was less than those of cattle, camel and human types. On the other hand, Refik *et al.*, (2002) showed that triglyceride level of the hydatid cyst fluids of human and sheep were higher than those of cattle type. Also, Sheep hydatid cyst fluid showed elevated level of triglycerides as compared to goats, cattle, camel and human (Shaafie *et al.*, 1999, and Izadi and Ajami, 2006).

Differences in biochemical composition of different hydatid cyst fluids suggest the possible existence of more than one strain of *E. granulosus* in human and other intermediate domestic animal hosts (Sharif *et al.*, 2004). Furthermore, also it had been indicated that the quantitative differences in the metabolism of *E. granulosus* and variations in biochemical composition of hydatid cyst fluids reflect strain variation in different intermediate hosts (McManus and McPherson, 1984; Thompson *et al.*, 1995 and Shaafie *et al.*, 1999).

Higher glucose level in hydatid cyst fluid and lower in protoscolices observed in the current study agree with those reported for hydatid cyst respective structures by Frayha and Haddad (1980).

From comparative point of view, in sheep hydatid cyst, both cyst fluids and protoscolices showed the highest glucose content. This was followed by goats and cattle. However, the glucose content of human hydatid cyst fluid was very close to that of the goat's hydatid cyst fluid. These results partly agree with those reported for hydatid cyst fluid from animals and human sources (Radfar and Iranyar, 2004). They found the lowest glucose level in cattle and human while the glucose content of sheep hydatid cyst fluid was slightly (but non-significantly) higher than that of the goats. Furthermore, the glucose content of infected liver and lung was about 33 % lower as compared with those of non-infected tissues. This may be due to the transport of glucose from the host tissues through the cyst membranes to the cyst fluid to support the nutritional requirements of the parasite.

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الخلاصة

تضمنت هذه الدراسة مقارنة بين المكونات الكيموحياتية للاكياس المائية (السائل العدري والرؤوس البدائية) إضافة الى الانسجة المصابة وغير المصابة المعزولة من كبد ورنثي الاغنام والماعز والابقار المذبوحة في مجزرة دهوك للفترة من تشرين الثاني 2009 الى نيسان 2010. إضافة الى عينات سائل الاكياس المائية المستأصلة من المصابين في مستشفى آزادي التعليمي في دهوك للفترة من آذار 2010 الى تموز 2010. احتوت الرؤوس البدائية لجميع المضائف على نسب عالية من الدهون الكلية والدهون الثلاثية والكولسترول مع ارتفاع ملحوظ في اكياس الماشية المصابة (63.81±1.434 ملغم/100غم و 38.28 ±1.277 ملغم/100غم و 28.85 ±0.795 ملغم/100غم على التوالي). وانسجة الكبد والرئة للماشية المصابة (57.78±1.3 ملغم/100غم و 51.91±1.299 ملغم/100غم على التوالي).

إحتوت السوائل العدرية على نسب عالية من سكر الكلوكوز في جميع المضائف مع ارتفاع ملحوظ لمستوى السكر في أكياس الاغنام (الكبد: 37.41±0.384 ملغم/ديسيلتر والرئة: 38.98±0.424 ملغم/ديسيلتر) والانسجة المصابة لرنث الاغنام (48.12± ملغم/100غم).

پوخته

دقی فہ کولینی دا، بہراوہر دکرہنکا بایو کیمیای ہاتہ کرن دناقہرا کیسکیٹ ئافی (ئافا کیسکا و سہرکیٹ دەستپیکی) زیدہباری پوشہکیٹ ئیشگری و بیٹ ساخلم بیٹ میلاک و پشیٹ پہز و بز و جیللیٹ ہاتینہ سہر ژیکرن ل سہرژینگہا دھوک دناقہرا چریا دووی 2009 ہہتا نیسان 2010. ہہروہسا چہند نموونہک ژ کیسکیٹ ئافی بیٹ کو ہاتینہ ژیفہکرن ژ نہخوشیٹ نہخوشخانا نازادی یا فیڈرکرنی دناقہرا ئادار 2010 الی تیرمہہ 2010. سہرکیٹ دەستپیکی بیٹ ہمی خانہخویا ئاستیٹ بلند دیار کرن ژ دونیٹ کوم و دونیٹ سیانی وکلوسٹولی، دگہل بلند بونہکا بہرچاف یا کیسکیٹ ئافی بیٹ جیللیٹ ئیشگری (63.81±1.434 ملغم/100غم و 38.28 ±1.277 ملغم/100غم و 28.85 ±0.795 ملغم/100غم د دویف ئیک دا). ہہروہسا پوشہکیٹ میلاک و پشیٹ جیللیٹ ئیشگری (57.78±1.3 ملغم/100غم و 51.91±1.299 ملغم/100غم د دویف ئیک دا).

ئافا کیسکا بلندبونہکا مہزن دیار کر ژ شہکرا گلوکوز دہمی خانہخویا دا دگہل بلندبونہکا بہرچاف ل ئاستی شہکری د کیسکیٹ پہزی دا (میلاک: 37.41±0.384 ملغم/ديسيلتر و پش: 38.98±0.424 ملغم/ديسيلتر) و پوشہکیٹ پشیٹ ئیشگری بیٹ پہزا (48.12± ملغم/100غم).