Effect of Aqueous Extract of Melia azedarach on the some Biochemical Parameters in the Liver Extract of Infected Mice by Lieshmania donovani

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Abstract

This study was designed to examine the effect of combined drugs (pentostam and allopurinol) 20mg / kg and 20,40,60,80 and 100 mg / kg of the aqueous fruit extract of M. azedarach on infected mice for 10,15, and 20 days to determine their effect on some marker enzymes such as aspartate amino transferase (AST) , alanine amino transferase (ALT) , alkaline phosphatase (ALP) , lactate dehydrogenase (LDH) and the biochemical parameters like glucose , cholesterol and total protein,

The present result showed a significant decrease of the two enzymes (AST and ALT) and significantly increased in (ALP and LDH) acivity in comparison with the enzyme activities in the liver of non– infected mice (con– ve). Where as the treated mice with 40 – 100mg / kg of aqueous fruit extract of the plant caused elevation in the activity of the (AST and ALT) and declining in (ALP and LDH) activity when compared with infected non – treated mice (con+ ve) after 10 ,15 and 20days post infection .

The results also showed, non – significant differences in the glucose and cholesterol concentrations in infected mice in comparison with the non – infected mice. The treated mice with 20 mg/ kg pentostam/ allopurinol and 40 – 100 mg / kg of aqueous fruit extract of the plant caused in significant elevation of the glucose and cholesterol concentrations in the liver of infected mice in comparison with (con+ ve) after 10 , 15 and 20 days post treatment , but the infection caused non – significant increased in the total protein in liver of mice in comparison with (con– ve) , the treated mice with 20mg / kg pentostam / allopurinol and 40–100 mg / kg from aqueous fruit extract M.azedarach caused decrease of total protein when compared with (cont+ ve) for 10 , 15 and 20 days post treatment .

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Introduction

Leishmaniasis is a disease caused by obligate intracellular parasites of the genus *Leishmania* which belong to the family Trypanosomatidae. They are dimorphic parasites presented as two principal morphological stages: the intracellular amastigote, within the mononuclear phagocytic system of the mammalian host, and the flagellated promastigotes within the

intestinal tract of the insect vector and in culture media [1,2].

Pentavalent antimonials, Amphotericin B , Miltefosine and allopurinol are drugs of choice have been successfully used for treatment of kala – azar,but requires a prolonged course of treatment it has been losing its efficacy in some regions and leads to serious side effects such as renal and cardiac toxicity [2,3,4,5,6].

The lack of an effective antileishmanial drug led to a renewed interest in the study of traditional remedies as sources for the development of new chemotherapeutic compounds with better activity and less toxicity. Natural products have potential in search for new and selective agents for treatment of important tropical diseases caused by protozoan. The plant kingdom is undoubtedly valuable as a source of new medicinal agents [7], alkaloids, terpenes, quinones, chalcones, lactones and saponins are known as antiprotozoal natural products found in higher plants [8].The linalo – rich essential oil from the leaves of *Croton cajucara* showed excellent inhibition of the growth of promastigote and amastigote forms of *L. amazonensis* [9] .

Materail and Methods

Organisms used:

Source of isolation:

A strain of *Leishmania donovani* (MHOM / IQ / 1982 / BRC1) is kindly provided by Nada Al – Bashir, College of Medicine / Al – Nahreen University, Baghdad. This stock culture was cultivated in Biphasic medium (NNN).

Exprimental animals:

Adult male BALB /c mice *Mus musculus*, 25 – 30 gm, were supplied by the animal house of College of Science / Mosul University, 6 – 8 weeks old were used for the pharmacological studies, and bred in the animal house of College of Medicine / Hawler Medical University. They were housed in controlled environment, the room temperature was maintained at 25 ± 2 C˚ and 10 hrs dark /14 light cycle with standard laboratory diet. Normal animals were kept under standard conditions, and used as controls.

Cultivation of parasites:

The stock culture of *L. donovani* used in this study according to Todie *et al* ., method [10] , was maintained successfully in the laboratory by weekly serial subculture.

Multiplication of parasites was done by inoculation of some of stock culture to new media after dilution with Lockś solution once each week. High numbers of parasites were maintained by cyclic subpassages *in vivo* in albino mice every three weeks, in order to maintain their virulence

Source and culture method of *L. donovani:*

L. donovani were kept in experimentally infected animals.The latter were sacrificed, the liver and spleen were removed aseptically in a hood. Samples were inoculated in a fresh NNN medium and kept at 26 C˚. Examination of these cultures was performed once a week for a period of 1 month.

Growth of organisms for experiments:

According to the method used by Alkhan [11] stock cultures of *Leishmania donovani* promastigotes were maintained on NNN medium at 26C˚ in McContry vials containing 5.0 ml of solid phase, slants overlaid with 3.0 ml of Lockś solution were inoculated with 0.1ml inoculums freshly isolated promastigotes from stock culture during their stationary phase. Subcultures were carried out every 5 days and third subcultures were used to infect the experimental animals.

Plant used:

The ripe fruits of *M. azedarach* were collected from plants of Arbil city during

autum. The plants were identified by herbarium staff, Biology department, College of Science, Mosul University and confirmed by referring to Issa [12].

The fruits were washed with distilled water after removing the dust

and debris, then dried in incubator at 37C˚ and stored in a dry place until processed.The fruit extracts were prepared according to Riose *et al.* [13].

Preparation of drugs:

Sodium stibogluconate (pentostam, Sb*)*

aqueous solution*:* Each ml of the solution containing 100 mg pentostam. The stock solutions were freshly prepared and sterilized using millipore filter 0.22µ and injected either intramuscularly (IM) or intraperitoneally (IP) . It was manufactured by the Wellcome Foundation, Ltd. England. It was stored at 4C˚, the stock was diluted by sterile distilled water, and the recommended dose for injection is 20mg / kg / day for 20 days.

Allopurinol (Urizol). Hpp.: Used for treatment of hyperuricemia, associated with chronic gout. It is manufactured by Welcome Foundation Ltd. England, stored at 25 C˚.

Each tablet of allopurinol contains 100 mg allopurinol, dissolved one tablet in 100 ml D. W. The concentration of the solution becomes 1mg / ml. The recommended dose of allopurinol for treatment of leishmaniasis was 20 mg / kg / day for 14 – 31 days, orally [14].

Experiments:

Seventy two male BALB/c mice 6 - 8 weeks old were used in these experiments. 63 mice were intraperitoneally infected with 100 × 10^6 promastigotes in stationary phase. The rest of mice were left without infection as control negative. The animals were divided into five groups as follows*:*

(1*)* The first group was consisted of 3 sub groups : 3 animals for each sub group and were used to evaluate the therapeutic effect of pentostam and allopurinol. Treatment with 20 mg / kg body weight / day was started 20days post infection. Each animal received 10 doses in the first sub group for 10 days. 15 doses in the second sub group for 15 days, and 20 doses in the third sub group for 20 days. Animals of these sub groups were sacrificed 10, 15 and 20 days post infection, respectively.

(2*)* The second group was consists of 3 subgroups (15 animals for each sub group) were used to evaluate the plant extract of *Melia azedarach*. Each animal received 10 doses in the first sub group, 15 doses in the second subgroup and 20 doses in the third subgroup .Each sub groups received five different doses of the extract 20, 40, 60, 80 and 100mg / kg body weight orally.

The animals of these sub groups were sacrificed 10, 15 and 20 days post infection respectively. The post necropsy procedure was followed as in the first group.

(3) The fourth group was consisted of noninfected untreated animals (n=9*)* which were left as a negative control group and sacrificed at 10, 15 and 20 days later and subjected to the same procedure previously mentioned.

(4) The fifth group was consisted of infected un – treated animals (n = 9*)*. This group served as positive control. On the10^{th}, 15^{th} and 20^{th} day the animals were sacrificed as in negative control.

Enzyme studies:

To check the activity of enzymes in the liver homogenate used : aspartate aminotransferase (AST) Henry method [15], alanine aminotransferase (ALT) Bergmeyer and Bent [16] method, alkaline phosphatase (ALP) Kind and King method [17] and lactate dehydrogenase (LDH) Henry method [18] .

For estimation of glucose concentration the Trinder method [19] was used. Enzymatic method which described by Allain [20] was

used for estimation of cholesterol concentration, and the colorimetric method described by Tietz [21] was used for estimation of total protein concentration.

Statistical analyses:

Results were analyzed by using Duncanś multiple range test which was used to compare the differences between the treatments.The probability of $P < 0.05$ was considered as significant [22] .

Results & Discusion

Tables (1) and (2) showed the levels of AST and ALTin infected mice, 72, 97.1 and 92.3 IU/L for AST and 35.1, 48.8 and 38IU/L for ALT lower than the levels in normal groups 112, 103.6 and 193.8 IU /L and 49.1,53.4 and 42.6 IU/L for 10, 15 and 20 days, respectively. The levels of these two enzymes in treated mice with combined drugs decreased in treated mice for 10 and 15 days were 86, 81.3 and 37.7, 38.1 IU / L compared with normal group 112, 103.6 IU / L and 49.1, 53.4 IU/L.But the levels of these two enzymes in treated mice with combined drugs for 20 days elevated to 140 IU / L. and 49.6 IU / L for AST and ALT respectively.

The tables (1) and (2) showed the levels of the two enzymes in treated mice with different concentrations of aqueous fruit extract of *M. azedarach.* The levels of two enzymes in treated mice with 20mg / kg from this extract were 83.7, 77.0 and 88.6 IU/L and 33.2, 45.2 and 39.6 IU/L which were lower than the levels in normal groups for 10,15 and 20days, respectively. The two enzyme levels elevated in treated mice with 40, 60, 80 and 100 mg /kg from the plant extract for the same period of time.

It is known that AST and ALT enzymes appears in liver cells, its lesser amounts in the kidneys, hearts and skeletal muscles . When the liver cells are damaged, resulting in abnormally high serum levels that may not return to normal for days or weeks [23].

The decline of AST and ALT values in infected mice with kala– azar, is due to using the liver extract in this study instead of serum, thus the AST and ALT released from the liver cells to the blood stream may caused decreased values of AST and ALT in liver cells.

Our study showed agreement with Pale *et al*.[24] , who revealed that *L. donovani* infection in hamsters significantly reduced the ALT and AST levels marginally.

The reduced activities of AST and ALT enzymes as found in this study might probably due to the presence of some compounds in aqueous extract like glycosides, myrosniz enzyme pectin and steroid compounds [25] thus, which may cause the inhibition of the parasites .

When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage [26]. Since the damage of liver cells happened due to the presence of the parasite *L. donovani* in it, this damage leads to leak of cytoplasmic enzymes into blood stream, so their activities are directed the liver cells and seems higher in the blood stream.

Tables (3) and (4) showed an increase in the activities of the enzymes of ALP and LDH of liver extract of infected mice were 18.2, 21 and 23.8 IU/L for ALP and 2250, 2060 and 2023 IU/L for LDH for 10, 15 and 20 days in comparison with the normal group 13.2,13 and 18.3 IU/L and 1780, 1648 and 1619 IU/L respectively. As shown in the tables (3 and 4) the increased ALP and LDH values in treated groups with combined drugs for 10 and 15 days were 25, 21.2U/L and 2023, 1890 IU/L respectively, but decreased values of both enzymes in treated mice with combined drugs for 20 days. This means that the low doses of combined drugs did not control the disease completely a result which confirmed by the appearance of promastigotes in inoculated cultures with the

liver of these groups. However the treatment for 20 days with 20mg / kg controlled the disease as the structure of the liver brought back to normal form.

Tables (3 and 4) showed the levels of the two previous enzymes in infected mice treated with different concentrations of aqueous extract of *M. azedarach.* The effect of 20mg / kg from the extract caused elevations of the levels of the two enzymes ALP and LDH 21.3, 17.9 and 33 IU / L and 2560, 2343 and 3300 IU / L for the period of 10, 15, and 20 days respectively, when compared with the normal groups. But the value of the two enzymes decreased by using different concentrations 40, 60, 80 and 100 mg / kg from the *M. azedarach* extract when compared with the normal groups. But the enzymes value decreased in treated mice with 60, 80 and 100 mg / kg for 10,15, and 20 days but did not lower than the normal, because these enzymes need long period of time not few days to return to normal values.

Our result showed an agreement with those of Pale *et al*.[24[who reorted that the level of ALP increased in infected hamsters with *L.donovani.* Furthermore, Rallis *et al*. [27] reported that ALP increased in hepatic tissue samples which were obtained from dogs with natural leishmaniasis caused by *L. infantum* . The present result is in agreement with the study of Akrawi [28] who reported that the LDH activity of kala – azar sera increased in cases of leishmaniasis when compared to those of normal sera, and also showed agreement with [29,30,31,32] as they confirmed that the serum LDH level was elevated in VL infection. The present findings also showed consistent with Tapisiz *et al*. [33] as they indicated that the elevation of serum LDH to 2762 IU / L, which is near from our result as the LDH in VL was 2250 IU / L (Table 4).

It can be suggested from the present study that the treatment of *Leishmania* with aqueous fruit extract of *M. azedarach* may be a new approach for sole or conjunctive therapy of kala – azar disease with minimal toxicity.

Tables (5 and 6) showed that the depletion of glucose and cholesterol levels in the liver extract of infected mice with VL were 92, 92 and 160.69 mg/dl for glucose and 21, 24 and 29 mg /dl for cholesterol in comparison with the normal groups 104, 112.8 and 177.08 mg/dl and 23, 28 and 32 mg/dl for a period of 10, 15, and 20 days respectively. While the levels of glucose and cholesterol increased in the treated mice with combined drugs for 10 and 15 days were 96 and 88 mg/dl for glucose and 20 and 24 mg/dl for cholesterol.

Tables (5 and 6) also show the concentrations of glucose and cholesterol in treated mice with aqueous fruit extract of *M. azedarach*, the decline of the glucose activity in the mice treated with 20mg / kg from the plant extract was 83, 61.9 and 117. 4 mg /dl for cholesterol and 17.1, 20.0 and 27.9 for glucose for 10, 15 and 20 days post infection. However, the treated mice with (40,60,80 and 100)mg / kg caused elevation of the levels of the glucose for the same period of time.This indicats that the extract of *M. azedarach* controlled the parasite and this result confirmed by the absence of promastigotes in cultures. This observation was supplement by histological examination of liver sections as the sections tend toward normalization.

Our results are consistent with Khalid *et al*. [34]who reported that the patients with leishmaniasis present low cholesterol levels and suggested that the activation of mononuclear phagocyte system explain those abnormalities. The results also concordant with Bekaert *et al*. [35] who investigated a reduced level of lipoprotein and cholesterol in the young children with VL. Liberopoulos *et al*.[36] reported that an old man with VL presented marked hypocholesterolemia and

severely decreased serum level of cholesterol.

Pucadyil *et al*.[37] reported that a significant reduction of cholesterol in the extended of leishmanial infection and the decreased concentration may be due to using of the plasma membrane cholesterol in process of internalization of the parasite to macrophage cells. Bansal *et al*. [38] suggested that the infected liver cells with *L. donovani* decreased the lipid / cholesterol that there may be some factors or enzymes, which allow the parasite to break up and consume lipid / cholesterol. This observation are consistent with our results which caused a decrease of the cholesterol levels in the infected liver mice.

Table (7) showed the increased value of total protein activity in the liver extract of increased value of total protein activity in the liver extract of infected mice were 3.6, 3.5 and 4.8 mg /dl when compared with the normal groups 3.4, 3.4 and 4.5 mg /dl for the period of 10,15 and 20 days respectively, and also the value increased in treated mice with combined drugs were 3.5 and 3.5 mg/dl for 10 and 15 days and 3.2mg/dl for 20 days in contrast with the normal groups.

Table (7) also showed that the levels of protein in the liver extract of mice infected with kala – azar disease and treated with the aqueous fruit extract of *M. azedarach*. The elevation of protein value in a dose of 20mg/kg from the extract were 3.8, 3.5 and 4.2 mg /dl when compared with the normal group. But the decline of the protein level in treated mice with 40, 60, 80 and 100 mg/kg from the extract in comparison with the normal group for the same period of time.This means that the *M. azedarach* extract in the doses 40,60, 80 and 100 mg / kg is considered as a good leishmanicidal agent that inhibit the parasite in the experimental mice.

Table (1): The effect of different concentrations of aqueous fruit extract of M. azedarach on liver enzyme AST activity(IU/ L) in liver extract of mice infected with L.donovani in comparison with pentostam and allopurinol , sacrificed at 10, 15 and 20 days post infection.

Duration / Treatments (mg/kg)	(Mean ± SE)of AST activity		
	10 th day	15th day	20 th day
Cont.-	112 ± 5.13 d	103.6 ± 3.76 c	193.8 ± 3.65** D
Cont. +	72 ± 2.54 b	79.1 ± 4.93*a	92.3 ± 4.89*** A
Pent. / ◌Allo	86.0 ± 0.83 c	81.3 ± 2.54*a	140 ± 13 B
Melia 20 mg/kg	83.7 ± 0.56 c	77.0 ± 1.63**a	88.6 ± 2.35*** A
Melia 40 mg/kg	111.82 ± 0.87d	99.3 ± 1.32***bc	174 ± 1.82***Cd
Melia 60mg/kg	115.5 ± 2.25 d	103 ± 5.03 c	178.7 ± 4.77**Cd
Melia 80 mg/kg	196 ± 4.62 f	110 ± 7.51* cd	186 ± 11.97 Cd
Melia 100 mg/kg	125.7 ± 3.76 e	120 ± 4.23 d	184 ± 7.03* Bc

*Means with different letters have significant difference at p<0.05 according to Duncan test.

* Significant differences of the f 10 day at p<0.05, ** at p<0.01 and *** at p< 0.001.

Table (2): The effect of different concentrations of aqueous fruit extract of *M.azedarach* on liver enzyme ALT activity (IU/ L)in liver extract of mice infected with *L. donovani* in comparison with pentostam and allopurinol, sacrificed at 10, 15 and 20 days post infection.

Duration / Treatments (mg/kg)	(Mean ± SE)of ALT activity		
	10 th day	15 th day	20 th day
Cont.-	49.1 ± 5.20 cd	53.4 ± 4.50 ab	42.6 ± 5.58 A
Cont. +	35.1 ± 5.97 ab	48.8 ± 3.12 ab	38.0 ± 9.17 A
Pent. / ◌Allo	37.7 ± 4.57 a-d	38.1 ± 1.87 a	49.6 ± 7.60 A
Melia 20 mg/kg	33.2 ± 0.83 ab	45.2 ± 0.72***ab	39.6 ± 0.51** A
Melia 40 mg/kg	36.6 ± 0.63 abc	50.0 ± 0.82***ab	40.8 ± 0.71** a
Melia 60mg/kg	37.7 ± 2.89 a-d	54.0 ± 4.08** b	42.0 ± 6.66 a
Melia 80 mg/kg	44.5 ± 5.46 bcd	57.1 ± 6.69* b	43.5 ± 3.67 a
Melia 100 mg/kg	50.6 ± 5.52 d	52.3 ± 7.97 ab	42.6 ± 8.47 a

* Means with different letters have significant difference at p<0.05 according to Duncan test .

* Significant difference of the 10 day at p<0.05, ** at p<0.01 and *** at p<0.001.

Table (3): The effect of different concentrations of aqueous fruit extract of M. azedarach on liver enzyme ALP (IU /L) in liver extract of mice infected with L. donovani in comparison with pentostam and allopurinol, sacrificed at 10, 15 and 20 days post infection.

Duration / Treatments (mg/kg)	(Mean ± SE) of ALP activity		
	10 th day	15 th day	20 th day
Cont.-	13.2 ± 1.27Abc	13 ± 4.08 ab	18.3 ± 3.98 a
Cont. +	18.2 ± 3.04a-d	21 ± 2.66 bc	23.8 ± 3.36 ab
Pent. / ◌Allo	25 ± 4.05D	21.2 ± 3.05 bc	20 ± 6.49* ab
Melia 20 mg/kg	21.3 ± 1.46a-d	17.9 ± 2.43 abc	33 ± 1.69** b
Melia 40 mg/kg	18.1 ± 2.67a-d	17 ± 2.31 abc	31 ± 2.49*** ab
Melia 60mg/kg	16 ± 4.43a-d	16.8 ± 3.56 abc	30 ± 3.57 ab
Melia 80 mg/kg	12.4 ± 3.14ab	13 ± 8.46 ab	30 ± 9.3 ab
Melia 100 mg/kg	11.7 ± 6.52 a	10 ± 2.5 a	22.9 ± 4.91 ab

* Means with different letters have significant difference at p<0.05 according to Duncan test.
* Significant differences of the 10 day at p<0.05, ** at p<0.01 and *** at p<0.001.

Table (4): The effect of different concentrations of aqueous fruit extract of M. azedarach on liver enzyme LDH (IU /L) in liver extract of mice infected with L. donovani in comparison with pentostam and allopurinol, sacrificed at 10, 15 and 20 days post infection.

Duration / Treatments (mg/kg)	(Mean ± SE) of LDH activity		
	10 th day	15 th day	20 th day
Cont.-	1780.0 ± 80.0a	1648 ± 177.43 ab	1619 ± 157.51 ab
Cont. +	2250 ± 141.66bcd	2060 ± 171.23 bcd	2023 ± 216.82 b
Pent. / ◌Allo	2023 ± 127.05 ab	1890 ± 86.94 bcd	1590.0 ± 166.56 ab
Melia 20 mg/kg	2560 ± 53.9 cd	2343 ± 52.29***d	3300 ± 105.36**c
Melia 40 mg/kg	2403 ± 118.29 bcd	2200 ± 182.23 cd	1990 ± 93.72** b
Melia 60mg/kg	2200 ± 184.19 abc	2116 ± 164.21 bcd	1670.0 ± 305.49*ab
Melia 80 mg/kg	2110 ± 402.87 abc	2009 ± 101.28 bcd	1619 ± 75.79 ab
Melia 100 mg/kg	2000 ± 122.35 ab	1233 ± 190.15**a	1214 ± 288.84* a

* Means with different letters have significant difference at p<0.05 according to Duncan test.
* Significant differences of the 10 day at p<0.05, ** at p<0.01 and *** at p<0.001.

Table (5): The effect of different concentrations of aqueous fruit extract of M. azedarach on the level of glucose (mg / dL) in liver extract of mice infected with L. donovani in comparison with pentostam and allopurinol , sacrificed at 10, 15 and 20 days post infection.

Duration / Treatments (mg/kg)	(Mean ± SE) 0f glucose conc.		
	10 th day	15 th day	20 th day
Cont.-	104 ± 11.79 a	112.8 ± 16.8 a	177.08 ±29.31 * b
Cont. +	92 ± 13.65 a	92 ± 16.85 a	160.69 ± 19.66** ab
Pent. / ♌Allo	96 ± 37.03 a	88.0 ± 30.09 a	167.1 ± 11.91 b
Melia 20 mg/kg	83 ± 17.04 a	61.9 ± 9.19 a	117.4 ± 11.07* a
Melia 40 mg/kg	85.3 ± 11.93 a	66.8 ± 4.90**a	130 ± 18.42* ab
Melia 60mg/kg	85 ± 23.64 a	113.5 ± 7.09 a	173.5 ± 18.21** b
Melia 80 mg/kg	87 ± 26.41 a	114.6 ± 13.91 a	167.86 ± 13.65* b
Melia 100 mg/kg	97 ± 4.85 a	115.09 ± 16.17* a	175.56 ± 16.47 b

*Means with different letters have significant difference at p<0.05 according to Duncan test.

*Significant differences of the 10 day at p<0.05, ** at p<0.01 and *** at p<0.001.

Table (6): The effect of different concentrations of aqueous fruit extract of M.azedarach on the level of cholesterol (mg / dL) in liver extract of mice infected with L.donovani in comparison with pentostam and allopurinol, sacrificed at 10, 15 and 20 days post infection.

Duration / Treatments (mg/kg)	(Mean ± SE) of cholesterol conc.		
	10 th day	15 th day	20 th day
Cont.-	23 ± 4.58 a	28 ± 4.26 b	32 ± 6.66* ab
Cont. +	21 ± 2.34 a	24 ± 6.16 ab	29 ± 5.6 ab
Pent. / ♌Allo	20 ± 4.97 a	24.0 ± 3.90 ab	28 ± 5.81 ab
Melia 20 mg/kg	17.1 ± 1.72 a	20.0 ± 4.18**ab	27.9 ± 4.25*ab
Melia 40 mg/kg	17.6 ± 1.37 a	27 ± 2.42* ab	29 ± 4.31 ab
Melia 60mg/kg	18 ± 3.86 a	28 ± 1.06 b	26 ± 5.51* ab
Melia 80 mg/kg	19 ± 4.58 a	28.6 ± 5.79 ab	24.6 ± 6.62 ab
Melia 100 mg/kg	24.6 ± 3.48 a	29.0 ± 1.80 ab	20.8 ± 4.61 ab

* Means with different letters have significant difference at p<0.05 according to Duncan test.

* Significant differences of the 10 day at p<0.05, ** at p<0.01 and *** at p<0.001.

Table (7) : The effect of different concentrations of aqueous fruit extract of M. azedarach on the amount of total protein (g / dL) in liver extract of mice infected with L. donovani in comparison with pentostam and allopurinol , sacrificed at 10, 15 and 20 days post infection.

Duration / Treatments (mg/kg)	(Mean ± SE) of protein conc.		
	10 th day	15 th day	20 th day
Cont.-	3.4 ± 0.68a	3.4 ± 0.71a	4.5 ± 0.65 ab
Cont. +	3.6 ± 0.51a	3.5 ± 0.63a	4.8 ± 0.32 ab
Pent. / Allo	3.5 ± 0.49a	3.5 ± 0.49a	3.2 ± 0.10 ab
Melia 20 mg/kg	3.8 ± 0.89 a	3.5 ± 0.72a	4.2 ± 0.09 ab
Melia 40 mg/kg	3.5 ± 1.17 a	4 ± 0.89 a	3.9 ± 1.02* ab
Melia 60mg/kg	3.4 ± 0.46 a	3.4 ± 0.75 a	3.4 ± 0.57 ab
Melia 80 mg/kg	3.4 ± 0.49 a	3.3 ± 0.67 a	3.4 ± 0.74 ab
Melia 100 mg/kg	3.3 ± 1.2 a	3.2 ± 0.7 a	3.2 ± 0.60 ab

*Means with different letters have significant difference at p<0.05

* Significant differences of the 10 day at p<0.05, ** at p<0.01 and *** at p<0.001.

References

[1] Roberts, L. S. and Janovy, J.(2005).“ Foundation of Parasitology”. 7th. Ed.,Singh, R.; Pandey, H. and Sunder, S.(2006).Visceral leishmaniasis (kala-azar). Indian J. Med. Res. 123: 331-44.

[2] Singh, R.; Pandey, H. and Sunder, S.(2006).Visceral leishmaniasis (kala-azar). Indian J. Med. Res. 123: 331-44.

[3] Sundar, S. and Rai, M. (2002). Advances in the treatment of leishmaniasis. Curr. Opin. Infect. Dis. 15 (6): 593-98.

[4] Ferreira, I. C.; Lonardoni, M.V.; Machado, G. M.; Leon, L. L.; Filho, L. G; Pinto, L.H. and Oliveira, A. J. (2004). Antileishmanial activity of alkaloidal extract from *Aspidosperma ramiflorum*. 1st Oswaldo Cruz, Rio de Janeiro. 99 (3): 325-27.

[5] Gupta, S.; Ramsh, SC. and Srivastava,VM. (2005). Efficacy of picroliv in combination with miltifosine an orally effective antileishmanial drug against experimental visceral leishmanianiasis. Acta Trop.94 (1): 41-7.

[6] Brenzan, M.; Nakamura, C.; Prado, B.; Ueda-Nakmura, T.; Young, M. and Cortez, D. (2007). Antileishmanial activity of crude extract and coumarin from *Calophyllum brasiliense* leaves against *Leishmania amazonensis*. Parasitol. Res. 101 (3): 715-22.

[7] Rocha, L G.; Almeida, J R.; Macedo, Ro. and Barbosa-Filho, J. M. (2005). A review of natural products with antileishmanial activity. Phytomedicine. 12 (6-7): 514-35.

[8] Wright, C. W. and Phillipson, J. D. (1990). Natural products and the development of selective antiprotozoal drugs.Phytotherap. Res. London. 4: 127-39.

[9] Rosa, M.; Mendonca-Filho, R.; Bizzo, H.; Rodrigus, I. Soares, R. and Loes, A. C. (2003). Antileishmanial Antimicrob. Agents Chemotherap. 47: 1895-901.

[10] Tobee,E.J., Brand , T.V.and Mehlmar , B. (1950). Cultural and physiological observations on Trypanosoma rhodesiense and trypanosome gambiense . J. Parasitol. 36: 48-54.

[11] Al-khan , H . I . (2001) . Effect of aqueous extracts of *Capparis spinosa* and

Citrullus colocysethis plants on growth and metabolism of *Leishmania major* promastigotes *In Vetro* .

[12] Issa, A. (1981). " Dictionare Des Noms Desplantes ". Dar AL-Raed, AL-Arabia. Beirut, Lebanon**.**

[13] Riose, J. L.; Recio, M. C. and Villar, A. (1987). Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. J. Enthopharmacol. 21: 39-52.

[14] Zgaeir, K. H.(1999). Allopurinol as anti leishmanial agent and its synergistic effect with sodium stibogluconate *In vitro* . Iraqi J. of Microbiology. 11(1) : 43-59.

[15] Henry, R. J. (1960).Method for L-Aspartate aminotransferase. Am. J. Clin. Path. 34: 381-98.

[16] Bergemeyer, H. and Bent, E. (1978). Method of enzymatic analysis. Clin. Chem. 24: 58-73.

[17] Kind, PRN. and King, EJ. (1954). Determination of serum alkaline phosphatase. J. Clin. Pathol. 7: 132-36.

[18] Henry, R. J. (1974). A comparison of serum versus heparinized plasma for routine chemistry tests. Am. J. Clin. Path. 61: 108-13.

[19] Trinder, R. (1969). Quantitative determination of glucose using GOD-PAP method. Ann. Clin. Biochem. 6: 24-7.

[20] Allain C. C. (1974). Enzymatic determination of total serum cholesterol. Clin. Chemother. 2(14): 470-75.

[21] Tietz, NW. (1995). Text book of Clinical Chemistry, 3rd ed. C. A. Curtis, E. R. Silveman, L. M. Christensen, R. H. P. 523-24.

[22] Steel, R.G.and Torie , J. (1980). Principles and procedures of statistics , 2nd ed., McGraw-Hill Book Company , Inc., London.

[23] Jaeger, J. J. and Denmark, H. H. (2002). Liver function and blood test. hepatitis C. Web site. H: /liver function tests htm.

[24] Pale, C.; Raha, M.; Basu, A.; Roy. K. and Gupta, A. (2002). Combination therapy with indolylquinoline derivative and sodium Antimony gluconate cures established visceral leishmaniasis in Hamsters. Antimicrob. agents and Chemotherap. 46 (1): 259 –61.

[25] Evans, W. C. (1997). " Trease and Evans ". Pharmacognosy. 4th ed. WB. Saunders Company. Asia PTE LTd.

[26] Nkosi, CZ.; Opoku, AR. and Terblanche, SE. (2005). Effect of pumpkin seed (*Cucurbia pepo*) protein isolate on the activity levels of certain plasma enzymes in Ccl_4-induced liver injury in low-protein fed rats. Phy.Ther. Res. 19: 341-45.

[27] Rallis, T.; Day, M.; Saridomichelakis, M.; Moraitou, K.; Papazoglou, L. and Fytianou, A. (2005). Chronic hepatitis associated with canine leishmaniasis. J. Comp. Pathol. 132 (2-3): 145 –52.

[28] Akrawi, B.A. (1985). Purification of LDH-1 and LDH-5 isoenzymes from kala-azaric sera and studies of their chemical and physical properties. M.Sc. Thesis, College of Science, Baghdad University.

[29] Wenzi, H.; Petritsch, W.; Decrinis, M.; Schreiber, F.; Warnkross, H. and Krejs, GJ. (1992). Kala-azar aquired in Croatia.Wien. Klin. Wochenschr. 104 (24): 757-60.

[30] Kawakami, A.; Fukunaga, T.; Usul, M.; Asaoka, H.; Noda, M.; Nakajima, T.; Hashimoto, Y.; Tanaka, A.; Kishi, Y. and Nmano, F. (1996). Visceral leishmaniasis misdiagnosed as malignant lymphoma. Internal. Med. 35: 502-06.

[31] Reus, M.; Garcia, B. and Vazquez, V.; (2005).Visceral leishmaniasis: diagnosis by ultrasound guided fine needle aspiration of an axillary node. Brit. J. Radiol. 78: 158-60.

[32] Dias, C.; de-Nazareth, M. M.; Eduardo, P.; Velloso, C. and Porrozzi, P. (2007). *Leishmania* chagasi: Cytotoxic effect of infected macrophages on parenchymal liver cells. Exp. Parasitol. 117 (4): 390-98.

[33] Tapisiz, A.; Belet, N.; Ciftci, E.; Ince, E and Dogru, U. (2007). Haemophagocytic lymphohistocytosis associated with visceral leishmaniasis. ESCMID 17_{th} European Congress of Clinical Microbiology and Infectious Disease. ICC, Munich. Germany, 31 March-04 April 2007.

[34] Khalid, F.; Bennaceur, Othman, H.; Achouri, E.; Ayachi, R. and Regaieg, R. (1990). Severe forms of liver involvement in visceral leishmaniasis. Arch. Fr. Pediatr. 47 (4): 257-60.

[35] Bekaert, E., Dole, E.; Dubois, D.; Bouma, M.; Lontie, J. and Jarrier, M. (1992). Alternations in lipoprotein density clases in infantile visceral leishmaniasis. Eur. J.Clin. Invest. 22 (3): 190-99.

[36] Liberopoulose, E.; Alexandridis, G.; Bairaktar,E.and Elisaf, M. (2002). Severe hypocholesterolemia with reduced serum lipoproteins in patient with visceral leishmaniasis.Ann.Clin. Lab. Sci. 32 (3): 305-08.

[37] Pucadyil, TJ.;Tewary, P.; Madhubala, R. and Chattopadhyay, A. (2004). Cholesterol is required for *Leishmania donovani* infection. Mol. Biochem. Parasitol. 133 (2): 145-52.

[38] Bansal, D.; Bhatti, H. S. and Sehgal, R. (2005). Lipid in health and disease. Bio.Med. Central. 4: 10-17.