European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 02, 2020

Recombinant Hsp70 Protein Fabrication And Testing For Immunostimulation

Marwa sami alwan[1] and Prof. Dr. Ghaidaa Abass Jasim[*1]

[1] Department of Biology, College of Education, University of Al-Qadisiyah, Iraq
[*1] College of Veterinary Medicine, University of Al-Qadisiyah, Iraq

marwa.alwan@qu.edu.iq, Ghaidaa.Abass@qu.edu.iq

Abstract

Heat shock proteins (HSPs) are a large class of proteins that have been conserved throughout evolution, present in all organisms studied so far, and are a major immunity in infections caused by diseases including leishmaniasis.

This study aimed to produce and develop the rhsp70 recombinant from *Leishmania major* parasite using advance gene synthesis technique and traditional recombinant methods. These proteins were then determined with the Western blot technique and testing the immune stimulation experimentally.

Groups of mice were injected with (rHSP70), and control the results were compared between those groups and between them with the positive and negative control groups, the results showed significant a difference between those groups, as the groups that were injected before infection with a concentration of 25 µg/ ml and 50 µg/ ml showed significant changes with all other groups The groups that were injected with a concentration of 75 µg/ ml and 100 µg/ ml showed significant changes with all groups except for the groups that were injected with a concentration of 75 µg/ ml and 100 µg/ ml after infection.

Introduction

(HSPs) are essential molecular chaperon to maintain cellular functions (Currie, 2011) by helping newly formed polypeptides to be folded into their correct protein shapes and avoid wrong folding of the protein (Banecka-Majkutewicz, Sawuła, Kadziński, Węgrzyn, & Banecki, 2012). It works to provide a link between the innate and acquired immune systems (Srivastava, 2002).

(HSPs) do an important role in protein homeostasis and can be found in all major cellular parts. It is expressed during cellular stress (hypothermia, oxidative stress, exercise, changes in pH, incorporation of new amino acids into proteins, viral infections, etc.), and it has been shown that some forms of environmental stress can induce HSPs response (Zininga, Ramatsui, & Shonhai, 2018).

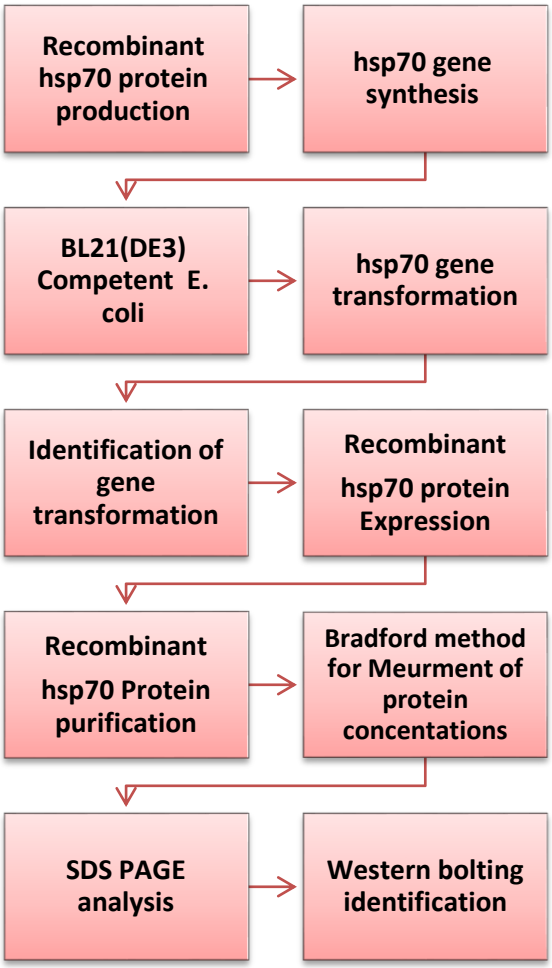
The HSP70 family acts as a molecular and reduces distortion results from stress and intracellular protein aggregation (Rokutan, 2000). HSP70 is also a member of the molecular descriptor family, which is involved in protecting cells against various types of stress. In addition, HSP70 has a protective role in tissue infection, its production in the liver and spleen grows as an acute phase reactor and is released into the circulation to facilitate disposal of dead cells (Merchant & Korbelik, 2011).

Material and Methods

Production of the recombinant heat shock protein hsp70 from *Leishmania major*

Recombinant *Leishmania major* hsp70 protein production

This study was designed to develop and produce the heat shock protein rhsp70 recombined from *Leishmania major* using advanced gene synthesis technique and traditional recombinant methods. These proteins were then determined by the Western blot technique as follows:



rhsp70 protein gene synthesis

The complete sequence of the hsp70 was synthesized using Advance next-generation gene synthesis technology which was installed in the cloning vector pET-32a (+) where the vector type was determined according to a method (Maharjan & Madhubala, 2015) that was prepared by the company. American GenScript, according to the following steps: The complete nucleotides sequence of the shock protein gene in *Leishmania* was obtained using the NCBI-Genbank site (XM_001684511.1). The whole nucleotides sequence of the shock protein gene in *Leishmania* was translated into the amino acid sequence to determine the effective coding region, depending on the open reading frame and the ExPASy translate tool. Online web site. The complete nucleotides sequence of the traumatic protein gene in *Leishmania* was sent to GenScript of America for synthesis in the plasmid cloning vector pET-32a (+) using HindIII and XhoI cutting enzymes by standard molecular cloning methods. The cloning was a recombinant of 6x His-tag within the pET-32a (+) vector to form the IPTG-inducible 6x His-tagged recombinant expression system and to show the rhsp70 *Leishmania major* _pET-28b (+) plasmid construct map. After the rhsp70 *Leishmania major* _pET-28b (+) clone gene was synthesized, a DNA sequencing test was performed to ensure the presence of the generated shock gene. Then *Leishmania major* _pET-28b (+) clone shock protein gene vector became

resistant to the Kanamycin antibiotic and ready for use with a suitable BL21 (DE3). Finally, 4µ of the aforementioned agglutinated shock protein gene was obtained from the American GenScript company and stored in deep freeze 80 - until it was used in the Gene transformation gene transfer step.

rhsp70 *Leishmania major* gene transformation

The clonal shock protein gene rhsp70 Leishmania major _pET-28b (+) clone was transferred to chemically competent *E. coli* BL21 (DE3) (C2527I) that was processed from British BioLabs, and the transfer method was performed according to the company's instructions.

Identification of gene transformation

Colonies of chemically transformed *E. coli* bacteria with cation that grew the LB agar plate with antibiotic ampicillin underwent PCR technique to detect expression of the recombinant hsp70 *Leishmania major* _pET-28b (+) express clone. Two of the primers in this study were designed for the rhsp70 gene and the carrier Competent *E. coli* isolate using the NCBI Genbank (AF527827.1) site (XM_001684511.1) and the primer design program Primer3 plus. These primers were provided by the Korean company Macrogen as in Table 1. Below:

Table 1: Molecular Diagnostic Gene Primers for PCR Assay

Primer		Sequence (5'-3')	Amplicon
16S rRNA gene competent E. coli	F	TGAGACACGGTCCAGACTCT	588bp
	R	GAGTTTTAACCTTGCGGCCG	
Recombinant hsp gene	F	CTGGTTCTGGCCATATGCAC	1991bp
	R	TGCTAGTTATTGCTCAGCGG	

Recombinant rhsp70 *Leishmania major* Protein Expression

Expression of the recombinant 6x His-tagged rhsp70 protein was performed using the BL21 (DE3) chemically competent *E. coli* expression system according to Westbye's method (Westbye, Fogg, & Beatty, 2014) in addition to using The QIAexpressionist ™ A handbook for high-. level expression and purification of 6xHis-tagged proteins. Fifth edition. 2003. QIAGEN. USA).

Recombinant rhsp70 *Leishmania major* protein purification

The instructions for extraction and purification of Recombinant 6x His-tagged rhsp70 protein extracted from soluble coliform bacteria were used according to the following method:

First: Soluble protein extraction

The soluble extracted protein was prepared from soluble coliform bacteria under native conditions as follows: The thawed granules cells coliform dissolved in the solution of the decomposition of the protein of the buffer Protein lysis buffer (Protein inhibitor enzyme cocktail Proteinase inhibitor cocktail) On ice for 15 minutes. Dissolved cells were distinguished by the ultrasound emitter apparatus Ultra Sonicator For (6X10secWith periods stop temporary 10 sec. at 200 - 300 watts) and the solution was preserved Lysates On snow during sonication Sonication. It has been conducting the process of expulsion central to the solution Lysates At 4 C° and 13000 cycle in the minute for 20 minutes. Then it was transferred material floating SupernatantAs an extract (A) Proteins are soluble and stored at -20 C°. The granules precipitated were suspended in a 5 ml extract of the buffer hydrolysis solution (B) Proteins is are soluble and stored at -20 C°.

Second : Purification of soluble 6x His-tagged rhsp70 protein

Has purified protein shock thermal soluble using the method Kromatokravaa Ni-NTA Agarose affinity chromatography methodAnd as the following figure 1.

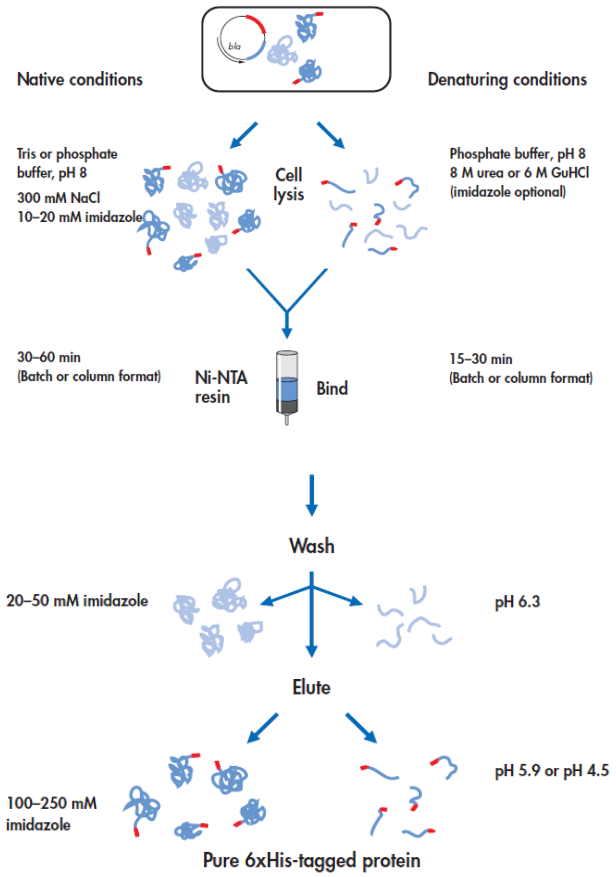


Fig. 1: Purification of a soluble heat shock protein using the Ni-NTA Agarose affinity chromatography method based on The QIAexpressionist ™ A handbook for high-level expression and purification of 6xHis-tagged proteins. Fifth edition. 2003. QIAGEN. USA) .

The purification of the soluble heat shock protein using the Ni-NTA Agarose affinity chromatography method is based on the ability to bind Ni-NTA agarose resins with the protein, for proteins that are expressed at high levels, about 10-50 mg of 6xHis protein were purified. -tagged protein per liter of cultured cells. As for proteins that are expressed at much lower levels, the purified ones were about (1-5 mg/ L). Lysis buffer contains Imidazole 10mM to reduce binding of untagged proteins and increase purification with fewer washing steps.

SDS PAGE electrophoresis technique

It is a technique that uses sodium dodecyl sulfate (SDS) and a polyacrylamide gel to separate protein molecules according to their molecular weight (kDa). The total crude protein extracted from the bacteria was analyzed, purified by separation column chromatography of 6xHis-tagged eluted hsp70 protein, which was analyzed with the above electrophoresis technique according to the method (He, 2011).

Proteins samples preparation

The protein samples were thawed on ice and about 15-20 micrograms were placed in a 0.2 ml tube with the addition of 2X Laemmli SDS Protein Loading Buffer (1-1) containing (375mM Tris.HCl, 9% SDS, 50% glycerol, 0.03%). bromophenol blue) and then adding 9% ß-mercaptoethanol (9% V/ V) as reducing agent. The mixing tubes were then placed in a thermal

block at 95C° for 5 min. to denaturation of protein folds and allowed to cool at room temperature for 5 min. After that the tubes were centrifuged for 5 min.

Samples loading and electrophoresis

The gel strip was removed from the molding holder and placed in the electrode set with a short pad inside and filled with 1x running buffer solution and the comb was carefully removed. Then 20 µl of protein sample and 5 µl of Protein Ladder Protein Ladder were loaded into the first hole using a long pipette tip. Then the electrode cover was closed on the Mini-PROTEAN Tetra Cell electrophoresis cell, and the power supply was operating in two times as follows:

a) 60 V for 30 minutes to stack protein folds.
b) 200 V for 35 minutes to separate protein bands.

Protein bands detection

After completion of the electrical runtime, the gel template was removed, the gel was carefully removed from the glass and washed twice with distilled water to remove the SDS buffer running buffer. The gel was then transferred to a Staining buffer solution container and placed in a rocker shaker for 24 h. Then, the gel was removed from the buffer staining solution that was washed twice with distilled water and transferred to a container containing Destaining buffer solution for at least 2 hours, or until the Coomassie Brilliant Blue dye was removed from the gel and only the protein bands appeared. Then the protein bands were examined on a white light viewer.

Western blot technique

The Western Plot technique was performed for the special detection of 6x His-tagged recombinant hsp70 proteins when the tag antibody reacted with the recombinant 6x His-tagged hsp70 proteins on the Nitrocellulose membrane. This technology was performed using the Western-Ready ™ ECL Chemiluminescence Substrate Kit from the US company BioLegend, and according to the manufacturer's instructions as follows: The membrane (from the top protein side) was incubated with 10 ml of ECL chemiluminescence substrate for 1-2 min. The final volume required is 0.125ml/ cm^2. The excess detection detector was removed and the spots were hidden, and any air bubbles were gently removed. The coiled spots (top protein side) were placed in the X-ray film and exposed to the X-ray. The exposure time varies from 5 seconds to 60 min.

Injecting laboratory mice

Negative control group

This group included (20) mice as a negative control group, and they were of different ages and genders. They were placed in special plastic cages that were fed with a special feed, and also provided with drinking water through special bottles with adequate temperature and ventilation.

Positive control group

This group included (20) mice, which were infected with *Leishmania* parasite. Where the isolate extracted from the cultures and which was isolated from the culture medium RPMI 1640 was injected, as it was injected with (0.2) ml of dilution of the sample taken from the culture medium with a density of (2 x 10^7 ml) parasite, by injecting it into the foot pad and in the ear fold and top Base of the tail.

Groups of mice treated with recombinant rhsp70 befor infection.

Each group (20 mice) was injected with purified recombinant rhsp70 protein at a concentration (100 µg/ ml, 75 µg/ ml, 50 µg/ ml, 25 µg/ ml) for each group at (0.1) milliliter subcutaneously with the use of a complete adjuvant, and after a week It was boosted by using

two doses of rhsp70 with an incomplete adjuvant for two weeks to produce antibodies against the recombinant protein rhsp70. Two weeks after the second booster dose, sera were collected for the ELISA test.

Treated groups after infection

After these groups were injected with purified recombinant rhsp70 protein at concentrations (100 µg/ ml, 75 µg/ ml, 50 µg/ ml, 25 µg/ ml) for a period of 3 weeks, in the fourth week this group was infected with the leishmaniasis parasite. Where the isolate extracted from the cultures was injected, which was isolated from the culture medium RPMI 1640, as it was injected with (0.2) ml of dilution of the sample taken from the culture medium with a density of (2 x 10^7 ml) parasite, by injecting it into the foot pad and in the ear fold and apex Base of the tail. And it was left for two weeks for the purpose of the appearance of the lesion and confirming the infection if it occurred, after which blood was drawn from it, and the serum was taken for an ELISA test to examine the antibodies.

Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA test was performed to quantitative detection of IgG antibody titers against experimental mice cutaneous *Leishmania* infection groups and recombinant Heat-Shock Protein 70 (rHSP70) vaccine groups. This test was prepared according to method previously described by (Rasouli, Hoseini, Kazemi, Abdolvahab & Kiany , 2009).

Results

Recombinant *Leishmania major* heat shock protein 70 (rhsp70) production results

Gene synthesis results

The recombinant *Leishmania major* heat shock protein 70 (rhsp70) expression production was dependent on successful of gene synthesis and correct open reading farm codons. In this study *Leishmania major* hsp70 gene complete nucleotide sequence was pick up from NCBI-Genbank (XM_001684511.1) as showed in figure 2:

The optimize function open reading farm (ORF) for hsp70 gene was identified by using ExPASy translate tool. Online web site and this correct open reading farm was showed in figure 3:

The optimize hsp70 gene sequence was synthesis by standard DNA synthesis technique by (GenScript. Company USA) with 100% sequence accuracy guaranteed. The synthetic hsp70 gene (Order name: U354REB060-2) was constructed in target cloning Vector pET-32a(+) by (HindIII/XhoI) restriction enzyme and tagged with N-Terminal polyhistidine-tag as showed in plasmid construction map figure 4.

The complete synthetic hsp70 pET-32a(+) clone was identified by digestion in restriction enzymes (HindIII/XhoI) and separated in 1% agarose gel electrophoresis as showed in figure 5.

The heat shock protein gene for *Leishmania major* heat shock Protein 70 (rhsp70) gene was synthesized using

primer(s) T7: (CTGGTTCTGGCCATATGCAC)

T7ter: (TGCTAGTTATTGC TCAGCGG)

the sequence product was analysis by NCBI BLAST Homology identity analysis and phylogenetic tree analysis to confirmed the complete constructed hsp70 gene sequence as well as submitted into NCBI database by Genbank accession number (XM_001684511.1)

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ATGACATTCGACGGCGCCATCGGCATCGACCTGGGCACGACGTACTCGTGCGTAGGCGTGTGGCAGAA
CGAACGCGTGGATATCATCGCGAACGACCAGGGTAACCGCACGACACCGTCGTACGTTGCGTTCACGG
ACTCGGAGCGGCTGATCGGCGATGCTGCAAAGAACCAGGTGGCAATGAACCCGCACAACACGGTGTTC
GACGCGAAGCGCCTGATTGGCCGCAAGTTCAACGACTCGGTTGTGCAGTCGGACATGAAGCACTGGCC
GTTCAAGGTGACGACGAAGGGTGACGACAAGCCCGTGATTTCGGTGCAGTACCGCGGCGAGGAGAAGA
CCTTCACGCCGGAGGAGATCAGCTCGATGGTGCTGCTGAAGATGAAGGAGACGGCGGAGGCGTACCTG
GGCAAGCAGGTGAAGAAGGCCGTGGTGACGGTGCCGGCGTACTTCAACGACTCGCAGCGCCAGGCAAC
GAAGGACGCCGGCACGATTGCTGGCCTGGAGGTGCTGCGCATCATCAACGAGCCGACGGCAGCGGCCA
TCGCGTACGGTCTGGACAAGGGCGACGACGGCAAGGAGCGCAACGTGCTGATCTTCGACCTTGGCGGC
GGCACGTTTGATGTGACGCTGCTGACGATCGACGGCGGCATCTTCGAGGTGAAGGCGACGAACGGCGA
TACACACCTTGGCGGCGAGGACTTCGACAACCGCCTCGTCACGTTCTTCACCGAGGAGTTCAAGCGCA
AGAACAAGGGTAAGAACCTGGCGTCGAGCCACCGCGCGCTGCGCCGTCTGCGCACGGCGTGCGAGCGC
GCGAAGCGCACGCTGTCGTCCGCGACGCAGGCGACGATCGAGATCGACGCGCTGTTCGAGAACATTGA
CTTCCAGGCCACCATCACGCGCGCGCGCTTCGAGGAGCTGTGCGGCGACCTGTTCCGCAGCACGATCC
AGCCGGTGGAGCGCGTGCTGCAGGACGCGAAGATGGACAAGCGCTCCGTGCACGACGTGGTGCTGGTG
GGCGGGTCAACGCGCATCCCGAAGGTGCAGTCCCTCGTGTCGGACTTCTTCGGCGGCAAGGAGCTGAA
CAAGAGCATCAACCCCGACGAGGCTGTCGCGTACGGCGCTGCGGTGCAGGCCTTCATCCTGACGGGCG
GCAAGAGCAAGCAGACGGAGGGCCTGCTGCTGCTGGACGTGACGCCGCTGACGCTGGGCATCGAGACG
GCCGGCGGCGTGATGACGGCGCTGATCAAGCGCAACACGACGATCCCGACCAAGAAGAGCCAGATCTT
CTCGACGTACGCGGACAACCAGCCCGGCGTGCACATCCAGGTCTTCGAGGGCGAGCGCGCGATGACGA
AGGACTGCCACCTGCTGGGCACGTTCGACCTGTCCGGCATCCCGCCGGCGCCGCGCGGCGTACCGCAG
ATCGAGGTGACGTTCGACCTGGACGCAAACGGCATCCTGAACGTGTCCGCGGAGGAGAAGGGCACCGG
CAAGCGCAACCAGATCACCATCACCAACGACAAGGGCCGGCTGAGCAAGGACGAGATCGAGCGCATGG
TGAACGACGCGATGAAGTACGAGGAGGACGACAAGGCACAGCGCGACCGCGTGGAGGCAAAGAACGGC
CTGGAGAACTACGCGTACTCGATGAAGAACACGCTCAGCGACTCGAACGTGTCCGGCAAGCTGGACGA
TAGCGACAAGGCCACGCTGAACAAGGAGATCGACGCGGCGCTGGAGTGGCTGAGCAGCAACCAGGAGG
CGACGAAGGAGGAGTACGAGCACAGGCAGAAGGAGCTGGAGAGCGTATGCAACCCGATCATGACCAAG
ATGTACCAGAGCATGGGCGGTGCTGCGGGCGGCATGCCCGGCGGCATGCCCGGCGGTATGCCGGACAT
GAGCGGCATGAGCGGTGGTGCAGGCCCGGCCGGCGGTGCGTCCTCTGGCCCCAAGGTCGAGGAGGTCG
ACTAA
```

Fig. 2: The complete *Leishmania major* hsp70 gene sequence (1991bp) that used for gene synthesis and cloned in Target Vector: pET-32a(+), Cloning site: HindIII / XhoI.

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MTFDGAIGIDLGTTYSCVGVWQNERVDIIANDQGNRTTPSYVAFTDSERLIGDAAKNQVAMNPHNTVF
DAKRLIGRKFNDSVVQSDMKHWPFKVTTKGDDKPVISVQYRGEEKTFTPEEISSMVLLKMKETAEAYL
GKQVKKAVVTVPAYFNDSQRQATKDAGTIAGLEVLRIINEPTAAAIAYGLDKGDDGKERNVLIFDLGG
GTFDVTLLTIDGGIFEVKATNGDTHLGGEDFDNRLVTFFTEEFKRKNKGKNLASSHRALRRLRTACER
AKRTLSSATQATIEIDALFENIDFQATITRARFEELCGDLFRSTIQPVERVLQDAKMDKRSVHDVVLV
GGSTRIPKVQSLVSDFFGGKELNKSINPDEAVAYGAAVQAFILTGGKSKQTEGLLLLDVTPLTLGIET
AGGVMTALIKRNTTIPTKKSQIFSTYADNQPGVHIQVFEGERAMTKDCHLLGTFDLSGIPPAPRGVPQ
IEVTFDLDANGILNVSAEEKGTGKRNQITITNDKGRLSKDEIERMVNDAMKYEEDDKAQRDRVEAKNG
LENYAYSMKNTLSDSNVSGKLDDSDKATLNKEIDAALEWLSSNQEATKEEYEHRQKELESVCNPIMTK
MYQSMGGAAGGMPGGMPGGMPDMSGMSGGAGPAGGASSGPKVEEVD-
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Fig. 3: The optimize function open reading farm (ORF) that showed the (frame 1) amino acids translate for hsp70 gene sequence by using ExPASy translate tool. Online web site.

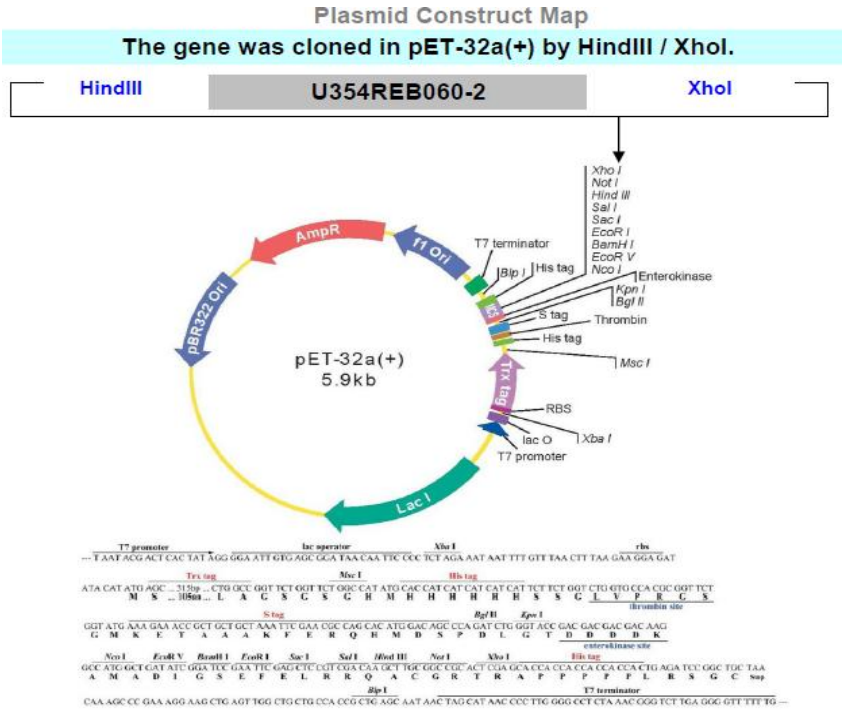

Fig. 4: The pET-32a(+) plasmid vector construction that showed the constructed hsp70 gene clone by using restriction enzyme HindIII/XhoI.

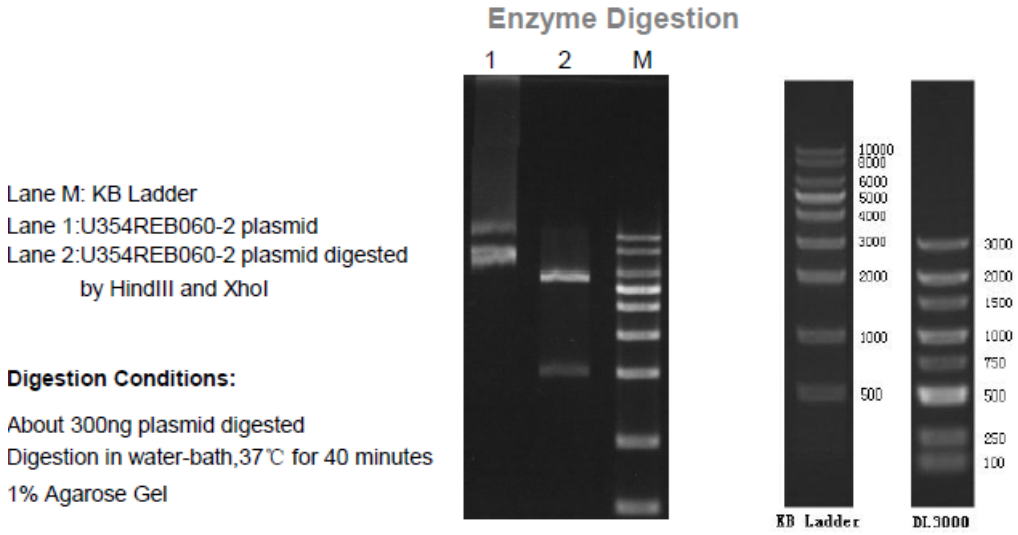

Fig. 5: The agarose gel electrophoresis image that showed 1991bp hsp70 pET-32a(+) clone that digested by restriction enzymes. This image was provided by (GenScript. Company USA).

Recombinant rhsp70 protein expression results:

The expression of recombinant rhsp70 protein was purified by Ni-NTA Agarose affinity chromatography technique. In this study hsp70 expression was optimized in (25°C) incubation temperature conditions by induction hsp70 protein was with (0.5mM IPTG, 0.75mM IPTG , and 1.0mM IPTG) for (4-5 hours). The concentration of purified rhsp70 protein was dependent on binding capacity of Ni-NTA Agarose into 6x His-tagged hsp70 protein by 10mM imidazole binding buffer . the results of rhsp70 protein expression was showed highest concentration

(924.89µg/ml) in second eluted tube by induction with (1.0mM IPTG) at 37°C for (4-5 hours), and less concentration was showed when induction at with (0.5mM IPTG and 0.75 IPTG) at 37°C. The concentration of eluted recombinant rhsp70 protein were measured by Bradford protein assay. The concentration of crude protein and eluted 6x His-tagged hsp70 proteins was measured by Bradford protein assay that dependent Bovine serum albumin (BSA) protein standard dilutes for standard curve calculation and results were showed in Figure 6.

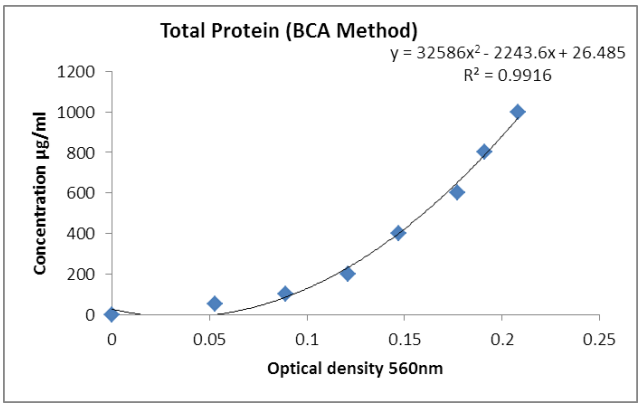

Fig. 6: The standard curve of diluted bovine serum albumin (BSA) protein concentrations.

ELISA results

The results of the current study showed a discrepancy between positive and negative control groups and between groups of mice that were injected with recombinant heat shock protein before and after infection with *Leishmania* parasite at 0.05. p As for the groups that were injected before the infection , the groups that were injected with concentration showed 25 µg/ ml And the 50 µg/ ml significant changes with all other groups showed that the groups injected with concentration 75 µg/ ml And the 100 µg/ ml Significant changes with all groups except those that were injected with concentration 75 µg/ ml and the 100 µg/ ml after infection , as shown in table 2 below:

Table 2: Demonstrates treated administration with recombinant heat shock proteinRecombinant rhsp70 protein

Groups		No.	Mean	SD
Negative control		20	0.395^{H}	0.069
Positive control		20	1.616^{A}	0.267
Groups of mice treated with recombinant rhsp70 befor infection	25 µg/ml	20	1.355^{C}	0.069
	50 µg/ml	20	1.007^{D}	0.061
	75 µg/ml	20	0.772^{F}	0.066
	100 µg/ml	20	0.696^{G}	0.069
Treated groups after infection	25 µg/ml	20	1.564^{B}	0.057
	50 µg/ml	20	0.922^{E}	0.054
	75 µg/ml	20	0.749^{FG}	0.060
	100 µg/ml	20	0.708^{FG}	0.116

Different letters denote to significant difference (P<0.05), similar letters denote to non-significant difference (P>0.05)

Discussion

The heat shock protein (HSPs) gene is a global response to stress. An organism or cells can be stressed by several factors, externally or internally. Ambient environmental factors can be among the stress factors and include extreme heat and cold, lack of oxygen, dehydration, stress, radiation and inflammatory processes. Like exposure to pathogens such as parasites, bacteria, viruses, and cancers, the role of these proteins is clear in stimulating the body's immune response to resist them, as these proteins are a strong stimulus for acquired and innate immunity (Zininga *et al*., 2018).

Heat shock proteins HSP70 works to resist the leishmaniasis parasite and this is done through association with phagocytes and T cells of various types, where the end result of the immune response is the elimination and destruction of the parasite (Milani *et al*., 2002), and this indicates the importance of HSP70 in the parasite's survival as long as the parasite is weakened. The immune system is healthy and effective.

Our study agree with the study conducted by (Figueiredo *et al*., 2009), which demonstrated that the HSP70 protein has the ability to stimulate the immune response of helper lymphocytes, as it stimulates both CD8 + Cytotoxity T helper cell) and (CD4 + T helper cell) cells. In another study by (Chen, Guo, Han, Yang, & Cao, 2009), it was found that the heat shock protein HSP70 released from heat-stressed cancer cells leads to the establishment of an anti-tumor immune response by stimulating the production of chemokines to the cancer cells and activating the dendritic cells via a path (TLR4).

And also from the studies that investigate the role of the protein (HSP70) in the immune system, the study conducted by the British researchers (Henderson & Pockley, 2010), in which they stated that the molecular accompaniments and wrapping proteins, intended for heat shock proteins (HSPS), are secreted by cells and act as special cellular signals Leukocytes have white blood cells and act as growth regulators that maintain the homoiostasis of the body's immune system.

Van der Auwera (Van der Auwera *et al*., 2013) identified *Leishmania* species on clinical samples based on the molecular sequence of the heat shock protein 70 (hsp70) gene, without the need for parasite isolation. In 2014, the scientist Ana *et al* scored a new goal for molecular diagnostics of leishmaniasis, as they assessed the utility of the gene encoding the heat shock protein (hsp) for the detection of leishmaniasis by means of a polymerase chain reaction (PCR). PCR was normalized and the analytical information was determined, as well as validity, diagnostics, accuracy and compatibility with PCR-18S, PCR-Hsp20 was obtained with DNA from a set of clinical samples from different sources, the analytical parameters were adequate .The sensitivity obtained was 86% and the specificity was 100%, and compatibility with the reference method was good (K = 0.731) supporting the possibility of using it for diagnosis and the possibility of subsequent identification of the species by means of the amplified product sequencing an additional advantage, and the utility of this gene as a new target for detection Leishmaniasis.

In addition, HSP is a strong innate stimulus in the immune response against pathogens that can be invested as antagonists in developing vaccines (Segal *et al*., 2006) and there are many studies that aim to design a future vaccine that protects against infection with Leishmaniasis parasites. A study of (Rasouli *et al*., 2009) in Iran and a study (Maharjan & Madhubala, 2015) in India.

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