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Mass Production of Bovine Serum Albumin

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Abstract:- Bovine serum albumin (BSA) extracted from the whole blood of cows and oxen from slaughter house in Thanlyin Township, Yangon Division during the study period from July 2004 to November 2007. Its lyophilized powder was prepared and their yield, purity and potency were tested. Polyacrylamide gel electrophoresis (PAGE) demonstrated the comparable purity of 10 % locally prepared BSA solution and imported BSA. Overall results in Enzyme- Linked Immuno Sorbent Assay (ELISA) showed that optical density (OD) values, for IgG and IgM (anticardiolipin) in normal and patient sera, were observed to be lower when local BSA was used compared to purchase commercial BSA.

Keywords:- *Bovine Serum Albumin, Lyophilized Powder, Polyacrylamide Gel Electrophoresis, Enzyme- Linked Immuno Sorbent Assay,*

I. INTRODUCTION

Albumin is generally regarded to mean serum albumin or plasma albumin. Albumin is used to describe a protein or a group of protein defined by solubility in water. Albumin is the most abundant protein in the circulatory system and contributes 80% to colloid osmotic blood pressure [1] .

Albumin is the most abundant and fairly homogenous protein of plasma. Approximately half of the total proteins of plasma is albumin. Albumin exerts low viscosity. It contributes 70 to 80 % of osmotic pressure and plays an important role in exchange of water between tissue fluid and blood[2]. Serum albumin is one of the most widely studied proteins and is the most abundant protein in plasma with a typical concentration of 5g / 100ml. It ischiefly responsible for the maintenance of blood pH [4]. Serum albumin is the carrier of fatty acids in the blood. Bovine serum albumin (BSA) binds free fatty acids, other lipids and flavour compounds, which can alter the denaturation of the protein. Isolated BSA has been reported to be a very functional protein. Denatured BSA might reduced the probability of a person acquiring certain diseases, such as insulin dependent diabetes or auto-immune disease[5] . Polyacrylamide Gel Electrophoresis (PAGE) is an extraordinary, flexible and versatile method for separation of proteins as well as nucleic acids. PAGE is used for separation and pruification of individual proteins and determination of molecular weight of purified polypeptides. PAGE is also the back-bone of the blotting technique[2]. Bovine Serum Albumin (BSA) is also used in immunological test for measurement of anticardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA). This is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.It is a useful and reliable method for clinical and experimental monitoring of patients with autoantibody related disease [3].

The objectives of this study are;

- To determine the amount of bovine serum albumin produced from bovine blood
- To determine the purity of bovine serum albumin by polyacrylamide gel electrophoresis
- To determine the potency of local bovine serum albumin by the measurement of anticardiolipin antibodies by ELISA

II. MATERIALS AND METHODS

A. Study site and study period

The present work was conducted in Pathology Research Division, Department of Medical Research (Lower Myanmar). Plate(1). The study period was from July 2004 to November 2007.

B. Collection of blood sample

Samples were collected from 42 cows and oxen from slaughter house in Thanlyin Township, Yangon Division.From each cow or ox 10ml of venous blood was collected in a sterile test tube.

C. Albumin preparation from blood sample

The serum was separated from whole blood by centrifugation at 2000 rpm for 10 minutes (Plate 2). In another test tube 0.3 ml serum was mixed with 4.5 ml of sodium sulphite. And then add 3 ml diethylether. Centrifuge at 2000 rpm for five minutes. After centrifuging, three layers appear in the test tube (Plate 3). The uppermost and middle layers were discarded. The uppermost layer is diethylether and middle is globulin layer. The lowest layer, the albumin layer, is collected into a separated tube. Sealed with paraffin wax and store at –80C for later use.

D. Lyophilization

Two ml bovine serum albumin was lyophilized (freezing followed by drying) into powder. It lasted 3 days for one batch. The albumin powder bottles were stored at –20.C deep freezer (Plate 4).

E. Bovine Serum Albumin Purity Test

The purity of locally produced bovine serum albumin and its lyophilized powder were examined by polyacrylamide gel electrophoresis (PAGE).

F. Bovine Serum Albumin Potency Test

The potency of local bovine serum albumin (BSA) was tested measuring anticardiolipin antibody by Enzyme–Linked. Immuno sorbent Assay (ELISA) according to [3].

G. Statistical analysis

The result from the test of local BSA and imported BSA in ELISA were subjected to t test analysis.

Plate 1:- Map of Showing Study site
Source:Google Earth 2019

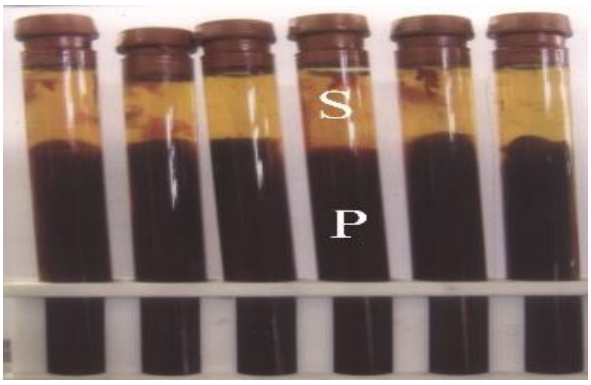


Plate. 2:- Serum separated from blood
(s=serum, p = precipitate)

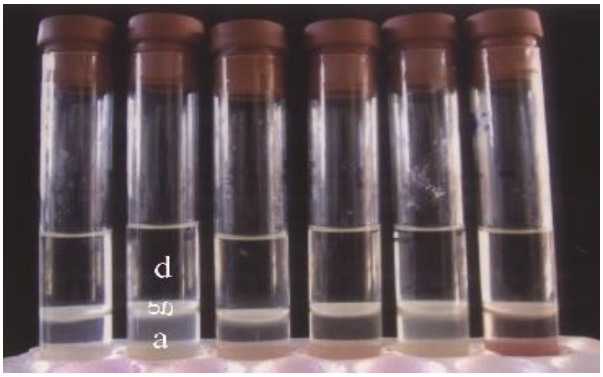


Plate 3:- Albumin separated from the diethylether and globulin by centrifugation
(d=diethylether , g =globulin , a =albumin)

III. RESULTS

A. Production of lyophilized powder from bovine serum albumin

Amount of bovine's blood collected, serum, albumin and lyophilized powder produced are shown in Table1, 2. Ten ml of collected blood yielded three-four ml of serum. The amount of albumin solution obtained was 2.5 to four ml from0.3 ml serum . One ml of albumin yielded 188.9 mg of lyophilized powder. Totally 15869 mg of lyophilized powder was produced from total blood volume of 420 ml. Mean lyophilized powder produced was 377.83 +/- 3.47 mg (N = 42).

Number of bovines	Amount of blood (ml)	Amount of serum (ml)	Amount of albumin (ml)
1	10	3.0	3.0
2	10	3.0	2.5
3	10	3.5	4.0
4	10	4.0	2.5
5	10	3.5	3.0
6	10	3.0	3.0
7	10	4.0	2.5
8	10	3.5	4.0
9	10	3.0	3.0
10	10	3.5	3.0
11	10	3.0	4.0
12	10	3.0	3.5
13	10	4.0	2.5
14	10	3.5	3.5
15	10	3.0	3.0
16	10	4.0	2.5
17	10	3.5	4.0
18	10	3.5	2.5
19	10	4.0	4.0
20	10	4.0	3.0
21	10	4.0	4.0
22	10	3.0	3.0
23	10	4.0	3.0
24	10	4.0	4.0
25	10	3.0	3.0
26	10	3.0	3.5
27	10	3.0	4.0
28	10	4.0	4.0
29	10	3.5	4.0
30	10	4.0	3.5
31	10	3.0	3.0
32	10	3.0	4.0
33	10	3.5	3.5
34	10	4.0	4.0
35	10	3.0	3.5
36	10	4.0	3.0
37	10	4.0	4.0
38	10	3.5	4.0
39	10	3.0	4.0
40	10	4.0	4.0
41	10	4.0	4.0
42	10	3.5	4.0

Table 1:- Amount of serum and albumin yielded from bovine blood samples

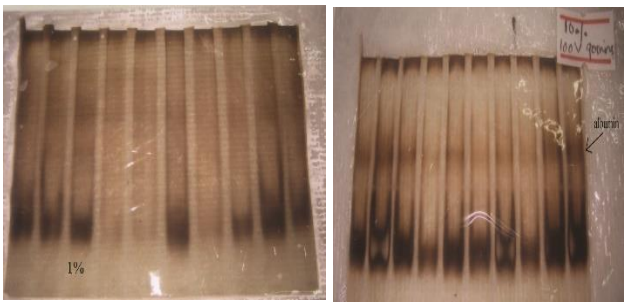
Number of bovines	Amount of bovine serum albumin (ml)	Amount of lyophilized powder (mg)
1	2	375
2	2	380
3	2	383
4	2	382
5	2	375
6	2	375
7	2	380
8	2	382
9	2	375
10	2	382
11	2	383
12	2	375
13	2	383
14	2	382
15	2	375
16	2	382
17	2	380
18	2	375
19	2	380
20	2	375
21	2	375
22	2	375
23	2	375
24	2	375
25	2	375
26	2	375
27	2	375
28	2	375
29	2	375
30	2	375
31	2	375
32	2	380
33	2	375
34	2	380
35	2	375
36	2	375
37	2	380
38	2	380
39	2	380
40	2	380
41	2	380
42	2	380

Table 2:- Amount of BSA lyophilized powder yielded from bovine serum albumin

Plate 4 Bovine serum albumin lyophilized powder

B. Polyacrylamide Gel Electrophoresis of locally prepared Bovine Serum Albumin

(Plate 5) shows electrophoretic separation pattern of serum albumin on Polyacrylamide Gel Electrophoresis. In 1% BSA solution, albumin band was not seen. In 10% BSA solution, only a single band was detected in polyacrylamide gel Electrophoresis. Other protein bands such as fibrinogen, globulin were not detected.



(a) (b)

Plate 5 Electrophoretic separation pattern of bovine serum albumin on polyacrylamide gel electrophoresis
(a) 1% BSA solution
(b) 10% BSA solution

C. Optical density of anti-cardiolipin antibodies Local bovine serum albumin test

➢ *IgG in normal controls*

In 1:100 dilution, optical density (OD) in normal controls detected by using locally prepared BSA is 0.355 and 0.532. optical density in normal controls detected by purchased BSA from foreign companies is 0.659 and 0.902.

➢ *IgG Level in Patients*

In 1:100 dilution, optical density (OD) in patients detected by using locally prepared BSA is 0.505, 0.538, 0.511, 0.528, 0.510 and 0.615. Optical density in patients detected by purchased BSA from foreign companies is 0.811, 0.791, 0.879, 0.740 and 0.740 (fig 1).

➢ *IgM Level in Normal Controls*

In 1:100 dilution, optical density (OD) in normal controls detected by using locally prepared BSA is 0.346, 0.369. Optical density in normal controls detected by purchased BSA from foreign companies is 0.436 and 0.518.

➢ *IgM Level in Patients*

In 1:100 dilution, optical density (OD) in patients detected by using locally prepared BSA is 0.343, 0.347, 0.340, 0.336 and 0.327. Optical density in patient detected by purchased BSA from foreign companies is 0.432, 0.449, 0.441, 0.429, 0.406 and 0.384 (fig 2).

Imported BSA was found to b highly significant , better than locally prepared BSA in antigenic effect.
IgG (t = -5.05,df =8 ,p <0.0005)
IgM (t = -6.18,df=8 ,p <0.0001)

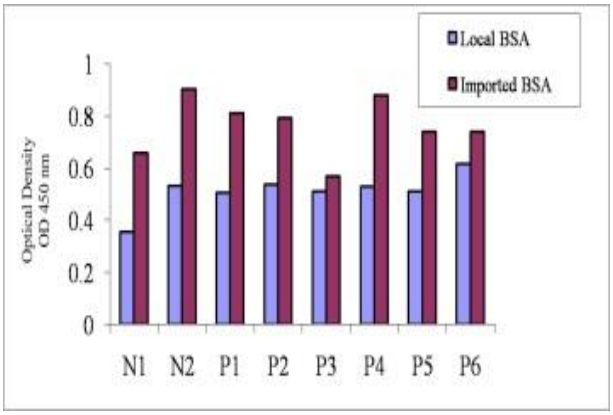


Fig. 1:- Optical density of local 10% BSA and imported 10% BSA for IgG (1: 100 dilution) (10% PBS/BSA, 1:100 dilution) IgG Level

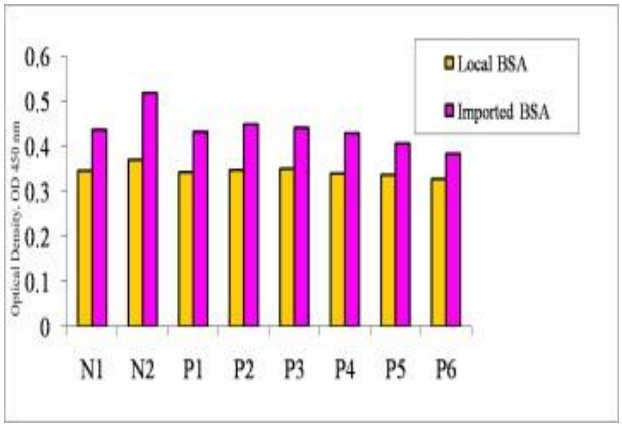


Fig. 2:- Optical density of local 10% BSA and imported 10% BSA for IgM (1 : 100 dilution) (10% PBS/BSA, 1:100 dilution) IgM Level

IV. DISCUSSION

The bovine serum albumin (BSA) and its lyophilized powder form are blood components of mammals useful for many research purposes such as protein separation for electrophoresis and for detecting immunoglobulins in blood samples by ELISA.

Currently BSA and its lyophilized powder are available from foreign companies and thus require foreign currency and import procedures to purchase for local research needs.

The present study accessed the feasibility of producing BSA lyophilized powder from local bovine blood serum and tested their purity and their effectiveness in immunoglobulin detection and quantification in blood samples in ELISA.

Polyacrylamide gel electrophoresis (PAGE) can be used to separate native proteins according to their change and size. The pattern of serum proteins on electrophoresis may be used in the diagnosis of disease. The purpose of the present investigation was to detect the protein bands in the bovine serum album in which is locally prepared. 10% BSA solution, only a single band is detected in polyacrylamide gel electropheresis 30%. Other bands (such as fibrinogen, globulin) bands are not detected. It indicates the locally prepared bovine serum albumin was composed of only albumin and almost free of globulin and fibrinogen. It seens that BSA has a high potential to be used in the laboratory practices.

The locally prepared BSA can be used in Enzyme-Linked Immunosorbent Assay (ELISA) for the determination of on antigen or antibody level in any body fluid.

Measurement of anti-cardiolipin antibody by ELISA using locally produced BSA lyophilized powder, blood samples from normal persons and patients, were collected and subjected to ELISA to detect their IgG and IgM profiles and measurements by OD at 450 nm wavelength. 10% local BSA in PBS was tested in comparison to imported BSA at 10% in ELISA to access the effectiveness of local BSA at 1:100 dilution in 96 well titer plate. BSA can not be used directly, so we can use with the ratio of 1:100 dilution.

Overall results showed although the use of local BSA in ELISA yielded IgG and IgM (anticardiolipin antibodies) counts in normal and patient blood sera, the OD values were always lower than that detected by purchased BSA from foreign companies. The reason may be due to less purification of the BSA and lesser concentration of BSA compared to purchased BSA.

V. CONCLUSION

The finding from this study will provide local production of BSA lyophilized powder from bovine venous blood might be highly useful and applicable in many research works in Myanmar.

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