5. Results

“We can’t solve problems by using the same kind of thinking we used when we created them.”

- Albert Einstein

(German born American Physicist. 1921 Nobel Prize for Physics. 1879-1955)

Serum is commonly used as a supplement in cell culture media. It provides a broad spectrum of macromolecule, carrier proteins for lipoid substances, trace elements, attachment & spreading factors, low molecular weight nutrients & hormones and growth factors. The most widely used animal serum supplement in cell culture is fetal bovine serum. Since serum in general is an ill-defined component in cell culture media, a number of chemically defined serum free media formulations have been developed in the last three decades. Besides the modern advantages in cell culture, their products for human welfare & in-vitro biological for therapies and considerable ethical concerns in collection of fetal bovine serum there is a pressing need for search of an alternate for FBS in the use of or partial replacement of serum with plant based products. This made the scientist to look for an alternate for FBS. In this angle this study also aimed to look for an alternate for FBS from plant source. For this study a total of three plant products viz., leaf gel of Aloe vera and seeds Cicer arietinum and Glycine max were chosen and tested for their serum replacement capability (Plate 1).

Amount of protein present in the selected plant product

As FBS is rich in protein and growth factor the protein content of the selected plant products were looked initially. Different concentrations of ammonium sulphate (20%, 40%, 60% and 80%) and cold acetone were used to precipitate the crude plant protein from their extract and their protein content was estimated and the same was shown in Fig. 5.1. Of the four different concentrations of ammonium sulphate used for precipitation, 60% was found to be efficient in precipitating most of the protein from the crude plant product extract i.e. aloe gel – 2.12 µg/ml, chickpea seed – 4.66 µg/ml and soybean seed – 8.47 µg/ml. Acetone was found to precipitate the protein still more better than ammonium sulphate i.e. aloe gel – 2.29 µg/ml, chickpea seed – 5.52 µg/ml and soybean seed – 10.38 µg/ml. The overall results revealed that soybean seed was found to have high concentration of protein of all the three
Plate 1: Photograph of Plant Parts Chosen

a) Aloe vera leaf gel

b) Chickpea Seed

c) Soybean Seed

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.1: Protein concentration of the plant parts chosen

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
plant protein chosen for this study. The cold acetone precipitation method was found to be best for getting total protein from plant products.

**Effect of plant extracts on Suspension cell culture**

*hPBMC*

To have an understanding about the growth promoting effect of the chosen plant proteins it was tried on normal hPBMC by dye exclusion method. It was found that the Complete Commercial Media (RPMI 1640 + 10% FBS) along with 5µg of soybean extract induced the proliferation of hPBMC (82 x 10^3 cells) better than chickpea (70 x 10^3 cells), *Aloe vera* (65 x 10^3 cells) and CCM alone (65 x 10^3 cells) (Fig. 5.2). In addition it was found that, as the time duration gets increased proliferation index was also goes higher.

As the addition of 5µg of the three plant protein extracts along with the complete commercial media (CCM) enhanced the proliferation of hPBMC better than CCM alone, the extent of supplementation was studied by adding the plant protein at various concentrations starting from 1µg/ml, 2 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml to the CCM.

When the activity of the three chosen plant proteins were assessed on hPBMC by SRB method it was found that proliferation of hPBMC increased with increase of plant protein content and reached maximum at 8 µg/ml for all the three plant proteins used. Of all the three, soybean protein enhanced the growth of hPBMC to an extent of 56%, followed by chickpea (53%) and *Aloe vera* (50%). This made it clear that addition of plant protein to CCM enhanced the growth of hPBMC more than that of CCM alone (Fig. 5.3). The results of MTT assay also confirmed the same i.e. 8µg/ml of plant protein added to CCM showed maximum cell growth (Fig. 5.4).

*Jurkat*

Other than normal hPBMC the growth promoting efficiency of plant proteins were checked on established cell lines like Jurkat (T cell cancer cell line) by SRB assay. Of the different concentrations of plant proteins tried cell proliferation was optimum at 8µg/ml of plant protein (Fig. 5.5), and soybean protein was found to be the best in promoting the growth of Jurkat (67%) than chickpea (58%) and *Aloe vera* (52%). The results of MTT assay also confirmed the same i.e. 8 µg/ml of plant protein supplementation was the best for Jurkat cell growth (Fig. 5.6).
Fig. 5.2: Effect of plant protein on hPBMC – Dye Exclusion Method

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.3: Effect of plant protein on cell growth (*in-vitro*) on hPBMC – SRB assay

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.5: Effect of plant protein on cell growth (*in-vitro*) on Jurkat – SRB assay

![Graph showing effect of plant protein on cell growth](image1)

Fig. 5.6: Effect of plant protein on cell growth (*in-vitro*) on Jurkat – MTT assay

![Graph showing effect of plant protein on cell growth](image2)

CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
**Raji**

Similarly when Raji (B cell cancer cell line) was cultured with plant protein supplement, it was found that all the three plant proteins showed maximum cell growth at the concentration of 8 µg/ml and soybean protein promoted the growth of Raji (38%) more than that of chickpea (33%) and *Aloe vera* (32%) (Fig. 5.7). Same kind of results were observed in MTT assay also (Fig. 5.8).

**Selection of plant based alternate for Fetal Bovine Serum**

Based on the concentration of protein and its effect on normal and established cancer cell growth, soybean seed was chosen for the further studies. Soy protein concentrate was prepared and purified to get soy protein isolate.

**Characterization and comparison of soy protein with fetal bovine serum**

As the aim of the study is to find a plant based alternate to fetal bovine serum a comparison was made between FBS and soybean protein in terms of its protein profile. Hence, molecular weight of the proteins present in both soybean and fetal bovine serum were done to have a basic understanding of their protein profile and it was observed that both FBS and Soybean showed the presence of albumin and globulin in them (Fig 5.9).

**Isolation of Protein**

SPI was subjected to column chromatography and different fractions were collected and their protein content was seen by UV-VIS spectroscopy at 280nm. The optical density (OD) values of different fractions were plotted as graph and the chromatogram was prepared. From the chromatogram it was observed that fraction number 2 to 7 had high protein (Fig. 5.10). The eluents with high protein content were pooled into one fraction, concentrated in a concentrator (Speed vac R plus 210A SAVANT) and was used for further studies. Characterisation of this pooled fraction by SDS PAGE showed the existence of different storage proteins like α, α’, β globulin and acidic & basic subunits of globulin protein (Fig. 5.11).
Fig. 5.7: Effect of plant protein on cell growth (*in-vitro*) on Raji – SRB assay

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture.
Fig. 5.9: SDS PAGE showing the soy protein and fetal bovine serum

Lane 1 & 2 : Fetal bovine serum albumin (G-Globulin, A-Albumin)
Lane 3 : Soy protein
Lane 4 : Molecular weight marker
   (Rabbit muscle myosin 205 kDa
    phosphorylase-b: 97.4 kDa
    Bovine serum albumin, 66.2 kDa
    Ovalbumin, 45 kDa; REase Bsp981, 25 kDa )

SDS PAGE of soy protein and fetal bovine serum, stained with Coomassie blue.

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.10: Spectrum showing the peak of different fractions of soy protein concentrate collected by Column Chromatography

![Graph showing OD at 280nm vs Fraction number](image)

Lane 1 - Pooled fraction of SPI
Lane M - Molecular weight marker containing Bovine serum albumin, 66.2 kDa; Ovalbumin, 45 kDa; lactate dehydrogenase, 35 kDa; REase Bsp981, 25 kDa.

Fig. 5.11: SDS PAGE showing the protein profile of soy concentrate

![SDS PAGE Image](image)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Separation of 7S and 11S protein fractions

To have better understanding about which of these fractions induce the growth of cells *in-vitro* SPI was subjected to further purification for its two important fractions like 7S and 11S.

The analysis of SPF7 and SPF11 on 12% SDS PAGE confirmed the presence of proteins like $\alpha$ (MW 63.17 kDa), $\alpha'$ (MW 58.06 kDa) & $\beta$ (MW 42.09 kDa) in 7S fraction and the acidic (MW 38.8 kDa) & basic (MW 21.04 kDa) subunits in 11S fraction. Both SPC and SPI also showed the presence of these proteins (Fig. 5.12).

Quantitative analysis of macromolecules in soy protein

To know about the nutritive value of soy protein the SPC and SPI were checked for their protein, aminoacid and carbohydrate content and it was found that the Soy Protein Isolate purified from the Soy Protein Concentrate had higher content of protein (67.83µg) and amino acids (71.33µg) and less amount of carbohydrate which was 19.52µg (Table. 5.1).

Effect of newly formulated culture media on suspension culture (Major media)

To know about, whether soy protein can be an alternate for FBS, culture media containing SPI as an alternate for FBS was prepared and was named as Soy protein isolate alternate media and were numbered serially based on the concentration of SPI added to it as SPIAM-1 to SPIAM-4. The concentration of SPI used in the media varies from 1mg/ml to 8mg/ml. Its effect was seen on the mammalian cell growth with human PBMC and established cell lines like Jurkat and Raji cell lines.

From the results it was found that, after 72 hours of incubation SPIAM-1 with 1mg/ml of SPI induced the growth of human PBMC ($65 \times 10^3$ cells) and it was more than that of SPIAM-4 with 8mg/ml concentration of SPI ($42 \times 10^3$ cells) (Fig. 5.13). This when compared with the results on the cells grown in CCM containing 10% FBS it was observed that CCM ($103 \times 10^3$ cells) is the best for human PBMC. The results of SRB assay supported the same i.e. SPIAM-1 promoted the growth to 57%, SPIAM-4 to 49% and CCM to 85% (Fig. 5.16). MTT assay and other proliferation assays like acid phosphatase, neutral red assay and BrdU assay also confirmed the same i.e. SPIAM-1 promoted the growth to 52%, SPIAM-4 to 46% and CCM to 78% (Fig. 5.17, Fig. 5.18, Fig. 5.19 and Fig. 5.20).
PLATE 4
Fig. 5.12: SDS PAGE showing the protein profile of SPC / SPI and its fractions like SPF7 & SPF11

SDS PAGE of soy protein, α, α’ & β subunit of the 7S fraction and acidic & basic subunit of 11S protein fraction, stained with Coomassie blue.

Lane 1: Bovine serum albumin
Lane 2: Soy protein fraction 11
Lane 3: Soy protein isolate (SPI)
Lane 4: Soy protein fraction 7
Lane 5: Soy protein concentrate (SPC)
Lane 6: Molecular weight marker (Bovine serum albumin, 66.2 kDa; Ovalbumin, 45 kDa; lactate dehydrogenase, 35 kDa; REase Bsp981, 25 kDa)
Table 5.1. Concentration of Major Nutrients in SPC & SPI

<table>
<thead>
<tr>
<th>Plant protein</th>
<th>Concentration of Major nutrients in SPC &amp; SPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total carbohydrate (µg)</td>
</tr>
<tr>
<td>SPC</td>
<td>33.89</td>
</tr>
<tr>
<td>SPI</td>
<td>19.52</td>
</tr>
</tbody>
</table>
An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture

Results

The same is the result on Jurkat cell line and Raji cell line (Fig. 5.14, Fig. 5.15, Fig. 5.16, Fig. 5.17, Fig. 5.18, Fig. 5.19 and Fig. 5.20) by all the six assays performed.

Overall this indicated that SPI alone may not be sufficient to enhance the cell proliferation and cannot be an alternate for FBS. Hence SPI was tried as a supplement for FBS in culture media.

Based on that, four different culture media were prepared with 10% FBS as a source of growth factor and four different concentrations of SPI viz. 1mg/ml, 2mg/ml, 4mg/ml & 8mg/ml respectively as supplement growth factor. These media were named as Soy protein isolate supplement media (SPISM-1 to SPISM-4) and their effect was tested on hPBMC, Jurkat and Raji cell line.

The results revealed that after 72hrs of incubation, the growth of hPBMC was comparable to that of CCM used in in-vitro culture i.e RPMI 1640 with 10% FBS. It was observed that there was an enormous increase in cell numbers in SPISM-1 (135x10^3 cells) and SPISM-2 (105x10^3) than CCM (98x10^3 cells). This indicated that in addition to FBS when SPI was given as supplement there was an enhancement in the growth of human PBMC (Fig. 5.21). The results of SRB assay supported the same i.e. SPISM-1 promoted the growth to 66%, SPISM-2 to 52% and CCM to 48% (Fig. 5.24). MTT assay, acidphosphatase assay, neutral red assay and BrdU assay also confirmed the same i.e. SPISM-1 promoted the growth of hPBMC to 73%, SPISM-2 and CCM to 71% (Fig. 5.25, Fig. 5.26, Fig. 5.27 and Fig. 5.28).

Similar is the results in Jurkat (Fig. 5.22) and Raji (Fig. 5.23, Fig. 24 and Fig. 25) by three methods performed. It was also noted that for both the cancer cell lines SPISM-2 was the best medium than SPISM-1 and CCM by acid phosphatase assay, neutral red assay and BrdU assay (Fig. 5.26, Fig. 5.27 and Fig. 5.28)

From the results it was understood that SPI can be a supplement for cell culture media.

Cell proliferation in SPIAM when analysed revealed that there is an inverse correlation between the concentration of SPI & the cell number, i.e. as the concentration of SPI found higher in cell culture media the viable cell count was decreased. Whereas in the presence of FBS, soy protein when used as supplement enhanced the proliferation of suspension cells better than that of FBS alone. This indicated that the soy protein alone may
SPIAM – Soy Protein Isolate Alternate Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.15: Effect of SPIAM on Raji – Dye Exclusion Method

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.16: Effect of SPIAM on suspension cells growth (in-vitro) – SRB assay

- SPIAM – Soy Protein Isolate Alternate Media
- CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

Fig. 5.17: Effect of SPIAM on suspension cells growth (in-vitro) – MTT assay
Fig. 5.18: Level of acid phosphatase in suspension cells grown on SPIAM (*in-vitro*)

SPIAM – Soy Protein Isolate Alternate Media  
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
SPIAM – Soy Protein Isolate Alternate Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
SPIAM – Soy Protein Isolate Alternate Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
not be an alternate to enhance the cell proliferation on its own and hence may not be a substitute for FBS in cell culture media. But it can be a supplement in cell culture media.

As the aim of the study was to find out an alternate for FBS in cell culture media an attempt was made to use different concentrations of SPI as replacement factor, reducing the level of FBS. Keeping this in mind another batch of media were prepared in which the concentration of FBS was reduced and was compensated with SPI. This media was named as Soy protein isolate replacement media (SPIRM-1 to SPIRM-4). This helped in finding out the optimum level of FBS replacement in media. The effect of this media was tried on human PBMC.

After 72hrs incubation it was found that SPIRM-3 with 5% of FBS and 4mg/ml of SPI promoted the growth of hPBMC to an extent of 6 fold in cell growth (Fig. 5.29), thereby indicating that SPIRM-3 could be the optimal media for hPBMC culture. The results of SRB assay revealed the same i.e. SPIRM-3 promoted the growth to 65% (Fig. 5.32). MTT assay also confirmed the same i.e. SPIRM-3 promoted the growth to 68%, (Fig. 5.33) along with the rest three proliferation assays performed like acid phosphatase assay, neutral red assay and BrdU assay (Fig. 5.34, Fig. 5.35 and Fig. 5.36).

Similar is the result on Jurkat cells (Fig. 5.30) and Raji cells (Fig. 5.31, Fig. 5.32, Fig. 5.33, Fig. 5.34, Fig. 5.35 and Fig. 5.36) by all the six assays performed.

As SPIRM-3 was found to be optimum for cell proliferation and to have a better idea of cell metabolism in the process, SPIRM-3 was analysed for metabolites like glucose, lactate, glutamine and ammonia. From the results it was found that cells grown in SPIRM-3 (RPMI 1640 + 5% FBS + 4mg/ml) used lesser glucose for its proliferation than SPIRM-5 (RPMI 1640 + 10% FBS) which consumed higher glucose during cell proliferation (Fig.5.37 anf Fig. 5.38).

From this it was understood that SPI can replace FBS to a certain extent and 50% FBS with 4mg/ml concentration of SPI could be the optimum for suspension cell culture in-vitro.

**Effect of newly formulated culture media on hPBMC (Minor media)**

SPI had two classes of storage proteins in them viz. the 7S globulin (conglycin) and 11S globulin (glycinin). To check their role on cell proliferation, two batches of media were prepared with 5% FBS and various concentrations of 7S and 11S fractions separately. These
Fig. 5.21: Effect of SPISM on hPBMC – Dye Exclusion Method

SPISM – Soy Protein Isolate Supplement Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.22: Effect of SPISM on Jurkat – Dye Exclusion Method

SPISM – Soy Protein Isolate Supplement Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.23: Effect of SPISM on Raji – Dye Exclusion Method

SPISM – Soy Protein Isolate Supplement Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.24: Effect of SPISM on suspension cells growth (in-vitro) – SRB assay

![Image of SRB assay graph]

CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

Fig. 5.25: Effect of SPISM on suspension cells growth (in-vitro) – MTT assay

![Image of MTT assay graph]

SPISM – Soy Protein Isolate Supplement Media

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture

Fig. 5.26: Level of acid phosphatase in suspension cells grown on SPISM (in-vitro)

SPISM – Soy Protein Isolate Supplement Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)
Fig. 5.27: Level of neutral red in suspension cells grown on SPISM (*in-vitro*)

**PBMC**

**JURKAT**

**RAJI**

SPISM – Soy Protein Isolate Supplement Media  
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.28: Level of BrdU in suspension cells grown on SPISM (*in-vitro*)

SPISM – Soy Protein Isolate Supplement Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
media were named as Soy protein fraction 7S replacement media (SPF7RM-1 to SPF7RM-4) and Soy protein fraction 11S replacement media (SPF11RM-1 to SPF11RM-4) respectively. When human PBMCs were cultured in these media and observed after 72hrs, maximum growth was seen in SPF7RM-3 (Fig. 5.39 & Fig. 5.40) and SPF11RM-3 (Fig. 5.41 & Fig. 5.42). It was found that 4mg/ml of SPF7 and SPF11 showed good growth and it was similar to 4mg/ml of SPI used in SPIRM. This indicates that the fractions either alone or in combination had the same effect on cell proliferation.

**Isolate and Identify Human Mesenchymal Stem Cells from Adipose tissue**

As the aim of the study is to see an alternate for FBS in stem cell growth, human mesenchymal stem cells (hMSCs) were isolated from adipose tissue and identified.

**Isolation of hMSCs from adipose tissue**

The cultured MSCs isolated from adipose tissue found to have fibroblast morphology (Fig. 5.43).

The morphology and internal organization of the adipose tissue was understood by observing the haematoxylin and eosin stained adipose tissue section. The cross section of the adipose tissue possessed the stromal vascular region in the centre surrounded by adipocytes. The stromal vascular cells include fibroblastic connective tissue cells, leukocytes, macrophages and pre--adipocytes. Several cells with small lipid droplets in the cytoplasm were also observed (Fig. 5.44).

The enzyme immunoassay using specific antibodies revealed the localisation of the mesenchymal stem cell surface receptor in adipose tissue section. When the sections were treated with secondary antibody conjugated with HRP followed by DAB, again the stromal vascular cells showed dark brown areas indicating the presence of MSC rich in CD29 and CD44 (Fig. 5.45).

The fluorescent immunoassay using specific antibodies also revealed the localisation of the mesenchymal stem cell surface receptor in adipose tissue section. After immunostaining, the vascular stromal cells of the adipose tissue showed the presence of mesenchymal surface markers CD29 & CD44 emitting fluorescence under fluorescent microscope (Fig. 5.46).
Fig. 5.29: Effect of SPIRM on hPBMC – Dye Exclusion Method

SPIRM – Soy Protein Isolate Replacement Media

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.30: Effect of SPIRM on Jurkat – Dye Exclusion Method

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.31: Effect of SPIRM on Raji – Dye Exclusion Method

SPIRM – Soy Protein Isolate Replacement Media

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
SPIRM – Soy Protein Isolate Replacement Media

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.34: Level of acid phosphatase in suspension cells grown on SPIRM (in-vitro)

SPIRM – Soy Protein Isolate Replacement Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.35: Level of neutral red uptake in suspension cells grown on SPIRM (in-vitro)

SPIRM – Soy Protein Isolate Replacement Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.36: Level of BrdU in suspension cells grown on SPIRM (in-vitro)

SPIRM – Soy Protein Isolate Replacement Media
CCM – Complete Commercial Media (RPMI 1640 + 10% FBS)

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
RT-PCR

Evaluation of ADSCs by RT-PCR confirmed that cells cultured from the SVF isolated from adipose tissue showed CD 90 & α-SMA, a MSC specific marker gene present in them thereby indicating the presence of undifferentiated ADSC gene (Fig. 5.47).

Effect of soy protein media on adherent culture

To determine whether soy protein isolate can support the mammalian cell growth, three adherent cells were used and are, Vero cell line, Adipose tissue derived stem cells (ADSC) and He La cell line. These were grown in culture media containing SPI as an alternate for FBS.

From the results of suspension cells 4mg/ml concentration of SPI with 5% (v/v) FBS was taken for the study of adherent cells with DMEM as basal medium.

With this, media was prepared for culturing adherent cells, that is A1 (DMEM + 5%FBS + 4mg/ml of SPI) which was compared with A0 (routein complete medium DMEM + 10% FBS), A2 (DMEM + 5%FBS + 5% commercial soy hydrolysate), A3 (DMEM + 5%FBS + 5% commercial serum replacement factor).

After 72hrs incubation it was found that A0 with 10% of FBS promoted Vero cells to an extent of $116 \times 10^3$ cells (Fig. 5.48) followed by He La cells ($103 \times 10^3$) (Fig. 5.49) and ADSC ($60 \times 10^3$) (Fig. 5.50).

From this study it was clear that the routein commercial media supported the growth of adherent cells thereby indicating that SPI and other serum replacement factor were not much effective than FBS in the medium. The results of SRB assay supported the same i.e. A0 promoted the growth of Vero cells to 72%, He La cells to 83% and ADSC to 78% (Fig. 5.51). MTT assay also confirmed the same i.e. A0 promoted the growth of Vero cells to 43%, He La cells to 48% and ADSC to 44% (Fig. 5.52).
Fig. 5.39: Effect of SPF7RM on hPBMC (*in-vitro*) – SRB assay

Fig. 5.40: Effect of SPF7RM on hPBMC (*in-vitro*) – MTT assay

SPF7RM – Soy Protein Fraction 7S Replacement Media

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.41: Effect of SPF11RM on hPBMC (in-vitro) – SRB assay

Fig. 5.42: Effect of SPF11RM on hPBMC (in-vitro) – MTT assay

SPF11RM – Soy Protein Fraction 11S Replacement Media

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.43: Isolation of MSCs from adipose tissue - Schematic representation

Processing of adipose tissue and isolation of adipose derived stem cells

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
PLATE 7

Fig. 5.44: Cross section of adipose tissue – H and E Stained

Cross section of human adipose tissue showing normal cellular architecture with distinct adipocytes and blood vessel rich in stromal layer.

- **SL** - Stromal layer
- **A** - Adipocyte
- **N** - Nucleus of adipocyte
- **C** - Cytoplasm
- **NC** - Nucleus of capillaries

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
PLATE 8
Fig. 5.45: Adipose tissue section showing CD29 (A) and CD44 (B) - Immunostained

A) CD 29

10x X 40x

Cross section of adipose tissue showing mesenchymal cell marker CD29 after immunostaining

B) CD 44

10x X 40x

Cross section of adipose tissue showing mesenchymal cell marker CD44 after immunostaining

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Adipose tissue expressing a unique set of CD markers after staining with fluorescent immunoprobe FITC

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.47: RT-PCR product expressing Mesenchymal stem cell markers

M - 20bp ladder.
Lane 1 - CD90 (121 bp)
Lane 2 - α-SMA (115 bp)
An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.49: Effect of formulated media on adherent cell line (He La)  
– Dye Exclusion Method

A0  - DMEM + 10% FBS  
A1  - DMEM + 5% FBS + 4mg/ml SPI  
A2  - DMEM + 5% FBS + Soy hydrolysate  
A3  - DMEM + 5% FBS + Serum replacement factor 3

A0 - DMEM + 10% FBS  
A1 - DMEM + 5% FBS + 4mg/ml SPI  
A2 - DMEM + 5% FBS + Soy hydrolysate  
A3 - DMEM + 5% FBS + Serum replacement factor 3

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.50: Effect of formulated media on ADSC - adherent cells
– Dye Exclusion Method

A0  - DMEM + 10% FBS
A1  - DMEM +  5% FBS + 4mg/ml SPI
A2  - DMEM +   5% FBS + Soy hydrolysate
A3  - DMEM +   5% FBS + Serum replacement factor 3
ADSC – Adipose tissue Derived Stem Cells

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture
Fig. 5.51: Effect of formulated media on adherent cells – SRB assay

A0 - DMEM + 10% FBS
A1 - DMEM + 5% FBS + 4mg/ml SPI
A2 - DMEM + 5% FBS + Soy hydrolysate
A3 - DMEM + 5% FBS + Serum replacement factor 3

ADSC – Adipose tissue Derived Stem Cells

An attempt to use Soybean protein as an alternate for Fetal Bovine Serum in animal cell culture