The use of animal derived compounds like FBS is essential for cell culture as they are rich in growth factors. But this may result in adverse side effects as they may carry infectious agents. The hazards to human health associated with the use of FBS are not still clearly identified. But it has been reported that the cells cultured in media with infected FBS lead to loss in the quality of the end product (Kallel et al., 2002). Hence there is an urgent need for an alternate to this animal derived compounds in cell culture. Efforts have been made to find an alternate for serum in culture media by substitute containing insulin, EGF, hydrocortisones and bovine pituitary extract (BPE) (Hammond et al., 1984). According to Kallel et al., (2002) BPE may still contain potential infectious agents. As there is no such problem in plant proteins they can be an alternate. Plant proteins do not have such infectious agents and hence there won’t be any adverse effects in the quality of the end product.

As FBS is rich in proteins, the search for plant based alternate to FBS was mainly focused on protein rich compound and plant products that has immunomodulatory property. Based on these criteria three plant products were taken as an attempt to find an alternate to FBS. In addition to the easy availability and low cost of plant origin serum replacements, another advantage is the increased safety. It is generally accepted that plant pathogens that might be present in plant derived biological products are not pathogenic to mammalian cells.

As the role of FBS in cell culture is its rich protein content and growth factors, the protein content of the selected plant products were looked initially. Soybean seed was found to have high concentration of protein of all the three plant products chosen for this study. This is in line with the earlier report of Wolf (1977), where it was documented that soybean is rich in protein concentration than that of chickpea and aloe gel. The reason for having high content of protein in soybean may be due to the accumulation of storage protein in the seeds of

“The there is no higher or lower knowledge, but one only flowing out of experimentation”

- Leonardo da Vinci
  (Italian Renaissance polymath, 1452-1519)
soybean. Earlier report revealed that nearly 40% of the dry weight of soybean is protein (Pringle, 1974) and the reason accounted is that the seeds are the organ of propagation and they need amino acids for their germination.

In the present study the protein content of soybean is found to be 42 µg/gm, whereas earlier reports stated that the protein content of soybean seed is twice as that of fat present and has reasonable balance of essential amino acids and the soybean seed act as a sink for surplus nitrogen with the amino acids like cysteine, methionine and sulphur containing amino acids (Shewry, 2008). Averyhart-Fullard et al., (1988) gave evidence for the presence of a soluble hydroxyproline rich protein in the soybean cell wall that is composed predominantly of five amino acids viz. proline, hydroxyproline, tyrosine, valine, and lysine. This could be a good reason for soybean seed extract to show higher proliferation rate.

As the aim of the study is to look for an alternate for FBS, particularly in relation to growth promoting efficiency, all the three plant product extracts were tried on the growth of normal hPBMC. Soybean promoted the growth of hPBMC better than the other two plant products, which may be due to its high protein content, growth factors and presence of lecithin. Lecithin is a phospholipid which is readily metabolised by human. Miranda et al., (2008) documented the role of soy lecithin i.e. supplementation of soy lecithin altered lymphocyte and macrophage function indicating it is an immunomodulatory compound.

Moreover proteins act as a carrier for different low molecular weight compounds and may facilitate cell proliferation. They are needed to protect the structural integrity of cell surface. According to Williams and his co-workers (1990), 5 to 30 kDa proteins act via cell surface receptors to generate signals, which stimulate cell proliferation. Eventhough some proteins do not take part in cell proliferation, their presence is required for the survival of the cells. Though the cells may be fully provided with nutrients and maintained under optimal culture conditions, deprivation of these proteins and peptides initiates apoptosis.

Aloe gel was found to have less concentration of protein among the 3 plant products chosen (Fig. 5.1). But according to Tizard and Ramamoorthy (2004) aloe gel was rich in compounds like major storage carbohydrate, acetylated mannan (acemannan) and pectin. Though the growth promoting efficiency of Aloe was found to be better than commercial media used for suspension cell culture in this study, it was less than that of chickpea and soybean. The reason could be the level of growth promoters in it. Tizard and Ramamoorthy...
(2004) have isolated acemanannan from aloe leaf gel and have reported that acemanannan acted as an immunostimulant.

The third plant product chosen for this study had a good quantity of protein i.e. 32.6µg/gm of seed, which was greater than that of aloe gel and lower than that of soybean, inferring that chickpea being a leguminous plant had the tendency to accumulate storage protein in the seeds (Fig. 5.1). When its effect on growth of human PBMC was tried, again it was better than aloe gel and lesser than that of soybean. The reason could be that, though chickpea belongs to legume family and had good quantity of protein it may not be rich in growth promoters essential for cell growth. In addition lower level of cell proliferating efficiency of chickpea may be due to the non-availability or reduced utilization of amino acids by the cells themselves. Earlier reports of Combe et al., (1991) supported this i.e. the methionine and threonine from chickpea was not fully available for metabolism. Girón-Calle et al., (2004) reported that chickpea seeds are a source of bioactive compounds and have proliferated the growth of J774 cells (macrophages).

As the initial addition of 5µg of the three plant protein extracts individually 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 (Fig. 5.3 & 5.4). The results revealed that the proliferation of hPBMC increased with increase of plant protein content and reached the maximum at 8 µg/ml for all the three plant protein used. This may be due to the fact that along with the high content of proteins, associated growth promoter quantity would have been more. Of all the three plant proteins used, soybean enhanced the proliferation of the cells more. Whereas at 16 µg/ml concentration, the chosen plant proteins inhibited the cell growth and the reason could be the fact that anything beyond the level may be toxic, i.e. higher concentration of amino acid or oligopeptides that are in the seeds might have disturbed the nutrients balance in culture medium. This result is in line with that of Zhang et al., (1994) where the growth of hybridoma cells were inhibited by the addition of higher concentration of protein. Lee et al., (2008) also found similar type of result on human keratinocytes.

When the same was tried on established cancer cell lines like Jurkat and Raji (Fig. 5.5 to 5.8) similar results were observed. This revealed that requirement of protein and other
growth factors are same for all the cells tried (hPBMC, Jurkat and Raji), which is something interesting in the sense that hPBMC is a normal primary cell; whereas Jurkat and Raji are cancerous cell lines. Theoretically the serum and other growth factors required for normal cells will be greater than that of cancerous cells, because cancer cells have the tendency to produce growth factors by themselves which in turn will be used for their growth. The results of Chun et al., (2007) proves this concept i.e. low concentration of protein was used to culture CHO cells, which are again a kind of cancer cell line.

Based on the above two results i.e. concentration of protein and its effect on normal and established cancer cell growth, soybean seed was chosen for the further studies. Hence a comparison was made between FBS and soybean protein in order to know about the protein profile and their similarities. The results revealed that both of them had albumin and globulin (Fig. 5.9).

Bovine serum contains two classes of proteins and are globulin and albumin. The SDS PAGE results showed the presence of 5 bands between 66kDA and 97kDa which indicate the presence of $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$ & $\gamma$ fractions. The rest of the bands with molecular weight lower than 66kDa represent the albumin fractions. Cann et al., (1949) also reported similar type of results with bovine serum. The soy protein showed two thick bands of proteins, one in the range between 66kDa to 97kDa and another around 44kDa. These two bands represent the 7S and 11S fractions of soybean protein. The 2S albumin fraction of soybean cannot be differentiated in SDS PAGE, as its molecular weight is similar to that of the basic subunit of 11S fraction. Earlier reports by Maruyama et al., (2003) and Özellikleri (2002) also revealed similar pattern of results with soybean.

Albumin is the major protein of serum and has several fractions that contribute to the growth and maintenance of the cells in culture. Albumin acts as the carrier protein of small molecules, particularly lipids. Transport of fatty acids is an important function of albumin, since these are essential for cells but are toxic in the unbound form and are also very poorly soluble in water. Albumin has specific binding sites for thyroxin and for metal ions like Ni$^{2+}$, Zn$^{2+}$ and Cu$^{2+}$. They also act as a buffer in maintaining pH and protect cells against damage by the shear force (Davis, 2002). It was proposed that albumin promoted growth by giving structural integrity to the cell membrane (Santucci et al., 2000).
The soybean 2S albumin (conglycinin) is reported to have an enzyme trypsin inhibitor. It is present in the form of dimer formed by the association of lysine and serine, where trypsin gets bound as it cannot cleave 2S albumin dimer, whereas it readily cleaves other molecules and helps in cell proliferation in cell culture (Shewry, 2008). The 7S globulin (β conglycinin) fraction contains 4 to 6 different subunits and about 6% of carbohydrate. The 11S globulin (glycinin) has two subunits i.e. the acidic subunit with 34kDa and the basic subunit with 19kDa. The acid subunit is proposed to have leucine, isoleucine and phenylalanine at their N terminal end whereas the peptides that are present in the basic subunit are rich in glycine in its N terminal end (Wolf, 1977).

After finding out the similarities between the profile of soybean and FBS, soy protein was taken for further studies and hence its concentrate was prepared (SPC) and further purified to soy protein isolate (SPI) by column chromatography (Plate 3). The purified form of the protein was analysed to ensure the protein profile so as to make use of the same for formulating various types of cell culture media. The biochemical analysis of two forms of soy protein purified showed that SPI had higher concentration of protein than SPC which may be due to the removal of insoluble carbohydrates. According to Weber (1981), SPI contains only the soluble proteins and sugars in them with low moisture content and high nitrogen solubility index.

The characterization of SPI and soy protein fraction (SPF) by SDS PAGE showed the presence of 7S fraction with their α, α’ and β subunits proving that it is a heteromeric protein and 11S fractions with their acidic and basic subunits. Study by Arrese et al., (1991) supported the same. It has been reported that 7S fraction contains 35% of soluble proteins and it is a mixture of some enzymes like heamagglutinin, lipooxygenase & globulins. The role of lipooxygenase in lipid oxidation and heamagglutinin is aggregation of cells in-vitro. 11S fraction contains 31% to 52% of soluble protein. It contains amino acids like leucine, isoleucine, phenylalanine and glycine. These proteins may play a vital role in cell proliferation (Bittencourt et al., 2007).

As soy protein enhanced the cell growth, an attempt was made to know which fraction of the soy protein i.e. 7S or 11S is involved in the proliferation and hence, the soy proteins were separated into its corresponding fractions. The results revealed the presence of
7S and 11S fractions in the corresponding molecular weight range. This is being reported by Bittencourt *et al.*, (2007) earlier in their studies.

After knowing the biochemistry of soybean and its purified products *viz.* SPC, SPI & SPF and their role on cell culture, various batches of cell culture media were formulated and prepared with different concentrations of soy protein prepared i.e. SPI, SPF7 and SPF11 as given in Table 1. The newly formulated media were coded as SPIAM – Soy Protein Isolate Alternate Media, SPISM – Soy Protein Isolate Supplement Media, SPIRM – Soy Protein Isolate Replacement Media, SPF7RM – Soy Protein Fraction 7S Replacement Media, SPF11RM – Soy Protein Fraction 11S Replacement Media. The concentration of SPI used in cell culture media was decided on the basis of its protein equivalent to FBS used in routine cell culture media.

The alternate media was designed in such a way that the media was devoid of FBS and instead of FBS, SPI was used as an alternate and hence the name alternate media. In the present study the cells didn’t proliferate in SPIAM when compared to CCM. The effect of alternate media when tried on hPBMC, Jurkat and Raji, it did not help the cells to grow appreciably when compared to CCM indicating that SPI cannot be an alternate for FBS. The probable reason could be the growth factors present in FBS may not be there in SPI. The inference is that SPI cannot replace FBS totally in cell culture media.

Hence an attempt was made to use SPI as supplement in a media which contains CCM & 10% FBS. So different concentrations of SPI were added as supplement (Table. 4.1) and SPISM were prepared and their effect on suspension cells *viz.* hPBMC, Jurkat and Raji were analysed. The results revealed that SPISM having 1mg/ml concentration of SPI along with 10% FBS in CCM induced the growth of cells to a maximum extent. On the other hand 1mg/ml concentration of SPI alone with CCM but devoid of FBS, did not promote the growth of cells appreciably.

In case of supplement media the FBS concentration was maintained at 10% and various concentrations of SPI was added. SPISM showed a good proliferation and the maximum cell proliferation was seen in SPISM-1 i.e. media supplemented with 1mg/ml of SPI.
All together it was noted that addition of 1mg/ml of SPI to RPMI 1640 without FBS (SPIAM) showed poor growth rate and when SPI was supplemented with FBS (SPISM) the cell proliferation was better. When the serum level was reduced and the SPI was supplemented (SPIRM) cell proliferation was better. This indicates that a minimum of 5% serum with 4mg/ml concentration of SPI resulted in good proliferation of PBMC.

Supplementation of cell culture media with SPI showed a good proliferation index in suspension cell culture, which clearly indicates that SPI is a good source of growth promoters and stimulate cell growth. This is similar with the previous report of Franèk et al., (2000) where rice and wheat hydrolysate were used as supplements with FBS. On the other hand, higher concentrations of SPI inhibited the cell growth in this study. The reason could be higher concentration of amino acids or oligopeptides present in plant protein. This in turn could have disturbed the nutrient balance. Based on that it was understood that SPI can be a supplement with FBS for suspension culture in-vitro, indicating the action could be synergistic to 10% FBS and 1mg/ml of SPI.

As the prime aim of the study was to formulate a medium devoid of FBS particularly for human application in terms of human stem cell therapy, another set of media was formulated and prepared by reducing the level of serum which in turn being replaced with SPI and this new set of media was designated as SPIRM and again their effect was tried on suspension culture in-vitro.

The results revealed that SPIRM-3 containing 5% FBS with 4mg/ml of SPI (which is equal to 5% FBS’s protein content) was found to be the best to hPBMC, Jurkat and Raji Cell lines.

It was interesting to note that reducing the level of FBS replaced by SPI induced cell proliferation better. From this study it was understood that a minimum of 5% FBS with 4mg/ml of SPI is a better option for hPBMC, Jurkat and Raji cell growth in-vitro. This indicates that when SPI was added as replacement along with FBS promoted the growth because of its protein content and other growth factors similar to FBS.

Earlier in 2000, Franèk et al., also showed similar results where rice and wheat hydrolate were used as supplement with FBS. Franèk and his co-workers have stated that natural peptides and plant protein hydrolysates had biological effect on the growth of
mammalian cells and their protein products. The reason could be that some peptides / proteins could interact with specific cell surface receptors thereby gives signal to promote cell growth, anti-apoptotic signals or protein biosynthesis and the effect is concentration based. This supported the current results of the study. These carbohydrate containing proteins have the ability to cause aggregation of cells in-vitro (Franèk et al., 2000). Yet another group worked on serum replacement media with chickpea hydrolysate (Girón-Calle et al., 2008) on THP-1 cells & Caco-2 cell lines. According to them chickpea hydrolysate containing medium favoured the growth of THP-1 cell lines. Chickpea hydrolysate promotes the growth of THP-1 cell lines (Suspension cell lines) is in line with the current results where suspension cell growth is promoted by SPI.

On the other hand when SPI level was enhanced & used as supplement along with FBS down regulated the cell growth indicating that anything beyond the level may be toxic to the cells. Additionally higher concentration of proteins, amino acids, oligopeptides present in SPI may be detrimental to the cells and would have caused the disturbance of nutrient in the media. There could be an antagonistic effect between FBS and SPI at higher concentration but synergistic at a concentration of 4mg/ml of SPI and 5% of FBS. Lee et al., (2008) reported that increasing the concentration of soy in medium decreased the cell proliferation which supports the present study. The low concentrations of SPI were able to sustain the proliferation of suspension cells.

Few earlier reports are found in support of this study, where plant products are tried as an alternate to FBS in cell culture. The plant proteins were used in the form of their hydrolysates.

Protein hydrolysates are enzymatically pre-digested for maximal speed of absorption by the cells. This additional processing often comes at an increased cost, but is considered to provide the nutritive equivalent of the original material in the form of its constituent amino acids (Silvestre, 1997). But in the present study proteins are used in its original form in order to know about the native function of the protein and the aim was also to formulate a plant based media free from serum and with cheaper cost.

The studies of Chun et al., (2007) showed that Bacto Soytone and soy hydrolysate were able to promote cell growth strongly and effectively in suspension cultures of CHO cells where the viable cell count was more. Later on, the same team tried a cell culture media with
few modifications and they investigated whether a safer plant product (soy) could replace BPE in the culture medium. Surprisingly their medium containing both BPE and soy protein hydrolysate (Bacto Soytone and Soy Hydrolysate) produced the largest number of viable cells, followed in descending order by the medium supplemented only with BPE, only with the hydrolysates and without supplementation (basal medium alone). They concluded that soy protein is an excellent source of nutrients for the growth of adherent cells (keratinocytes), although they do not fully substitute for BPE.

Similar work was performed by using chickpea hydrolysate on THP-1 cell line and Caco-2 cell lines by Girón-Calle et al., (2008), and reported that chickpea hydrolysate promoted the growth of THP-1 cells but did not allow the growth of Caco-2 cells.

It was interesting to note that Kannan et al., (2009) made an attempt to use Tualang honey as a supplement with fetal bovine serum in cell cultures and found cell proliferation. They could see the inverse correlation between honey concentration and cell proliferation indicated by the mitotic index values of osteoblast cell lines after 48 hours. They have also reported the non-toxic nature of honey inferred by no chromosomal aberrations in honey treated CRL1543 cells.

Another group led by Sagirkaya and his co-workers (2004) showed that FCS can be replaced with synthetic serum supplement in the in-vitro maturation medium. This was necessary to obtain a higher cleavage rate and development rate to the blastocyst stage of immature bovine oocytes recovered from ovaries obtained from slaughter houses.

After knowing the fact that soy proteins isolate can not only reduce but also replace FBS in suspension cell culture, an attempt was also made on adherent cell culture, particularly MSCs of adipose tissue origin in-vitro. As the prime aim is to develop an alternate for FBS in regenerative therapy i.e. stem cells it was isolated and characterised first.

A stem cell is an unspecialized cell that is capable of replicating or self-renewing itself and developing into specialized cells of various cell types. Under the right conditions the stem cells divide to form more cells called daughter cells. These daughter cells can either become new stem cells (self-renew) or specialized cells (differentiation) with a specific function. Based on the source of origin, stem cells are of two types. They are embryonic and adult stem cells (Bongso and Lee, 2005). The primary role of stem cell is to maintain and repair cells in living organisms. Most of the works done on adult stem cells are focused mainly on mesenchymal stem cells (MSC) which are non-hematopoietic cells that too of bone marrow
(BM) stromal origin. The disadvantage of bone marrow stem cell is the cost of its isolation & potential morbidity in donors during isolation (Noort et al., 2003). The other sources of adult stem cell could be umbilical cord blood (Shetty et al., 2007), chorionic villi of the placenta (Abumaree et al., 2012), amniotic fluid (Carraro et al., 2008), peripheral blood (Campagnoli et al., 2001), fetal liver (Chou and Lodish, 2010), lung (Kajstura et al., 2011), adipose tissue (Cawthorn et al., 2012) and even in exfoliated deciduous teeth and dental pulp (Wang et al., 2012).

The major source of MSC taken in the study was adipose tissue. The stromal vascular fraction of the adipose tissue was taken for stem cell isolation. To have a clear understanding about the structure of adipose tissue histology was performed. From the histology of the AT it was clear that AT has a stromal vascular region.

There are evidences that adipose tissue is derived from mesoderm and contains a stromal vascular cell population. Adipose tissue was used as negligible and as simple filling tissues. The evolution in the discovery of stem cells indicated that they are not only composed of adipocytes but also of other cell types found in the stromal vascular fraction It is reported to contain non-adipocytes such as preadipocytes, endothelial cells, pericytes, monocytes, macrophages and stromal stem cells (Keating, 2012). Added to this is the reports of Gimble et al., (2007) reporting that, adipose stromal vascular fraction has macrophages, fibroblast, blood cells, endothelial cells and precursor cells for adipose tissue. These stromal cells have pinned the concept of adipose tissue plasticity and saying that adipose tissue is considered as a reservoir of stem cells.

Adipose tissue displays higher blood perfusion rate than many other organ and is extensively vascularised. This vascularised area was found to be a rich source for adult stem cells (Cinti, 2005) and contains a higher stromal region which is reported to have stem cells.

Barrilleaux and his co-workers (2006) were able to differentiate adipose tissue derived stem cells into bone, cartilage, fat and muscle. These cells of fibroblast morphology were later called as mesenchymal stem cells. Adipose tissue derived stem cell also has a fibroblast like morphology similar to MSCs derived from other tissues. A comparative analysis of MSC derived from bone marrow, adipose tissue and umbilical cord made by Kern et al., (2006) showed that ADSC were similar in morphology, immune phenotype, rate of isolation & differentiation capacity.
This study demonstrated that stromal cells derived from subcutaneous adipose tissue have the properties of mesenchymal stem cells. The tissue, when digested with collagenase separated the adipocytes. Upon separation by centrifugation the stromal vascular cells settled down at the bottom. When cultured in a suitable medium like DMEM containing 10% FBS, the stem cells in the stromal vascular fraction adhered on to the plastic surface and acquired a fibroblast-like morphology. 80% confluence was observed in a week. These cells were screened for MSC markers by RT PCR and found positive for CD90 and SMA.

Characterization of MSCs was reported using the expression of cell-specific proteins and CD markers (Conget and Minguell, 1999; Pittenger et al., 1999). Like MSCs, expression of CD29, CD44, CD71, CD90, CD105/SH2, and SH3 marker and absence of CD31, CD34, and CD45 was also showed by Zuk et al., (2001). Proteins that were consistently expressed by a majority of ADSCs (average 97% or more of cells) included HLAABC and CD29 (integrin β1), CD49 (integrin α5/VLA-5), CD51 (integrin αV), and CD90 (Thy-1). Our study showed the presence of the markers like CD29, CD44, CD90 and α-SMA consistently that too in the absence of CD45 differentiating adipose derived stem cells from hematopoietic lineage.

The present study screened for markers at two ways a) directly on the tissue sections confirming the presence of MSC in the tissue itself and b) in the cultured ADSC by RT PCR. The mesenchymal stem cell markers used in this study were, i) CD29, a MSC specific marker called as β-integrin. This marker is responsible for adhesion of stromal cell in culture. Only when the cells starts adhere on the substratum they show fibroblast morphology. ii) CD44 is also known as hyaluronate which is a MSC specific stromal antigenic marker. iii) CD90, also called as Thy-1 is responsible for the formation of extra cellular matrix and iv) α-SMA is responsible for the cytoskeleton formation.

A recent article in Japan reported the efficient differentiation of CD29 murine ADSC into cardiomyocytes like cells based on morphology, molecular and protein expression profile (Yamada et al., 2006). The expressions of α-SMA in the in-vitro culturing of ADSC was reported earlier by Lee et al., (2006) and Gagnon et al., (2002).

Undifferentiated ADSCs transcribe many genes that are related to the extracellular matrix and angiogenesis which are implicated in inflammation, morphogenesis, and tissue repair. Highly transcribed genes include endoglin; FGFs; FGFR3; neuropilin-1; osteonectin; fibronectin; VEGF-D; TGF-β R2 and R3 as determined by RT-PCR (Katz et al., 2005).
also we could see the expression of CD90 (Thy-1), a mesenchymal stem cell specific adhesion molecule. CD90 is expressed in MSCs during the time of development and the results confirmed the presence of MSCs in adipose tissue.

After characterizing ADSC, adherent cells like Vero, HeLa cell lines and the isolated ADSC were cultured in the newly prepared plant protein based media. The effect of SPI was compared with commercially available soy hydrolysate and serum replacement factors. The results showed that the complete commercial medium with 10% of FBS alone is good for adherent cell culture. This may be due to the reason that adherent cells are more critical and very fragile to culture conditions; adaptation by adherent cells to a new medium may need more time. According to Van Der Valk et al., (2010) to make a new plant based medium suitable for adherent cell in the culture the following ways can be tried i.e. i) growing the cells in a mixture of new and original medium with the gradual increase in the proportion of the new medium or ii) direct transfer to the new medium with a transitory period of decreased viability and slower growth rate. The first option seems to be better for some media, but apparently does not work for others (Rajala et al., 2007). As the current study applied the direct transfer of cells to the newly prepared medium which caused prolonged doubling time, potentially leading to selective pressure.

Van Der Valk et al., (2010) in his report stated that, typically, a cell culture has to undergo a gradual weaning process which involves progressive adaptation to lower serum concentrations until serum-free conditions are reached. The cultures to be adapted should be in the logarithmic phase of growth and should have viability over 90%. This is in support of the present study as the adherent cells first need to attach themselves over the surface of the substratum for cell proliferation and hence the stress for the adaptation to newly prepared media might have reduced the proliferation rate.

Yet another study by Girón-Calle et al., (2008) reported a similar kind of report to the present study, where chickpea hydrolysate was used as an alternate for FBS which supported the cultivation of THP-1 (suspencion cell) and was not effective on Caco-2 (adherent cell). They also states that this might be due to the presence of residual amount of non-peptidic bioactive compound that might inhibit the growth of Caco-2 cell line.

Contradictory to the present finding Kunova et al., (2010) reported successful cultivation of human embryonic stem cells and maintenance of the same in undifferentiated
state on commercially available plant product VegetaCell (hydrolysate from gluten of spelt wheat). Initially the human embryonic stem cells used in their study was maintained in DMEM/F12 with KSR as an alternate to FBS. After the confluence has attained they were transferred to vegetacell media along with human serum albumin, 250x cholesterol lipid concentrate, ascorbic acid, human insulin and sodium selenite. From this report it may be clear that if still more work and changes are made in the formulation of the present media with SPI and ITS supplement (insulin. Transferrin and selenium) SPI can also be an alternate to FBS for adherent cell growth in-vitro.

Van Der Valk et al., (2010) recommended to start a new formulation with a 50:50 (v/v) mixture of DMEM and Ham’s nutrient mixture F-12. This medium formulation combines the high amino acid content of DMEM with the highly enriched Ham’s F-12. Furthermore, the basal medium must contain an essential, so called, ITS supplement (insulin, transferrin and selenium). Insulin, the first of the components of the ITS supplement, now the most commonly used hormone in culture media. Transferrin is also an essential protein in culture medium where the main action is to transfer iron into the cells (Bjare, 1992). Selenium is an essential trace element and acts in selenoproteins which protect cells against oxidative stress (Helmy et al., 2000).

With time and reports, it is clear that almost every cell type has its own requirements concerning medium supplements. Therefore, a universal (serum-free) cell and tissue culture medium may not be feasible. Different cell types have different receptors involved in cell survival, growth & differentiation and release different factors to their environment. But still the main aim of researchers is to formulate a new media free of animal product for the wellbeing and safety of human health.