

## Putative Prophylaxes Updated of Placenta Extract and *Aloe vera* as Biogenic Stimulants

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### ABSTRACT

Placental tissue has been discarded post partum, but it is now becoming recognized as a potential source of stems and progenitor cells. Updated findings were shown in the present review that this tissue holds visible promise as a source of stem cells which have widespread clinical applications. Defibrotide (a polydeoxyribonucleotide fraction) from human placental extract could reduce incidence of hepatic veno-occlusive disease which is a leading morbidity and mortality after haemopoietic stem-cell transplantation. KiSS-1 is a human metastasis suppressor gene that inhibits the metastasis of human melanomas and breast carcinomas, without affecting tumorigenicity. KiSS-1 encodes a carboxy-terminally amidated peptide with 54 amino-acid residues. The peptide was isolated from human placenta as the endogenous ligand of an orphan G-protein-coupled receptor and termed 'metastin'. Metastin has potential as a suitable biomarker and novel effective therapy for cancer metastasis. JBP485, a novel dipeptide isolated from human placental hydrolysate, was recognized as a target reagent in drug-drug interaction and to restore liver function *in vivo* study. Cognitive decline in elderly people often derives from the interaction between aging-related changes and age-related diseases, such as diabetes, and covers a large spectrum of clinical manifestations, from intact cognition through mild cognitive impairment and dementia. *Aloe vera* supplementation may provide the efficacy of hypoglycemic activity as well as lowering lipid oxida-

tion, and the possible putative prophylaxes to optimize cognitive decline in older adults with pre-diabetes symptom. Because of the diversities of placental extract and *Aloe vera* as biogenic stimulants, present review introduced the briefly selected authors' intentions in each topical subject. Specially, the influence of gut microbiota to host is astonishing and highlighting that the consequence of our behaviours affect not only the environment without, but also that within us. Based on geriatric and neurological viewpoint, present review highlights current therapeutic strategies of placenta extract and *Aloe vera* as the so-called "biogenic stimulants", aimed at producing a biological rejuvenator in placental extract and improving cognitive function via modifying the brain's insulin sensitivity in *Aloe vera*.

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**Key words:** Placenta extract and *Aloe vera*; Biogenic stimulants

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### INTRODUCTION

Aging is considered to be one of the most complicated and heterogeneous phenomena and is the main risk factor for most chronic diseases, disabilities and declining health. Over the years, a number of anti-aging medicines of natural and synthetic origin have been introduced. Epidemiological and experimental data indicate that diet plays a major role in the pathogenesis of many age-associated chronic diseases, and in the biology of aging itself. Data from human studies indicate that long-term calorie restriction (CR) with adequate intake of nutrients results in several metabolic adaptations that reduce the risk of developing type 2 diabetes, hypertension, cardiovascular disease and cancer<sup>[1]</sup>. Moreover, CR opposes the expected age-associated alterations in myocardial stiffness, autonomic function, and gene expression in the human skeletal muscle<sup>[2]</sup>. Sarcopenia is the age-related loss of muscle. It leads to loss of muscle power, which

in the end results in frailty and disability. In general, it appears that 5-13% of persons aged 60-70 years and 11-50% of persons in their 80s have sarcopenia. There are numerous causes of sarcopenia<sup>[3]</sup>. Changes in muscle mass and quality might play a central role in the pathway linking malnutrition, its biological and molecular consequences, and function.

Several interventions have been explored in the last years to counteract the age-related muscle decline. These include protein supplementations, physical exercise, testosterone replacement (as well as other anabolic androgens) in men, estrogen replacement in women, growth hormone replacement, and treatment of vitamin D deficiency. To date, adequate protein intake and resistance training are the most promising interventions able to prevent and/or delay the decline of muscle mass and function<sup>[4]</sup>. Epidemiologic evidence suggests that some types of diet, such as the Mediterranean diet, might prevent negative functional outcomes in older adults<sup>[5]</sup>. However, despite a theoretical and empirical basis, intervention studies using nutritional supplementation have shown inconclusive results in preventing functional impairment and disability.

Human placental tissue is generally available from secundina at birth, and powdered dried placenta (Zihe Chi, in Chinese) has been used for treatment of renal diseases, together with human hepatic and pulmonary diseases as a restorative and rejuvenation kampo-medicine. Injection of human placental suspension, however, may produce serious undesirable side effects, such as an allergic-like shock and viral infections. Thus, potential benefits are expected for clinical treatment with placental hydrolysate. As earlier methods for the administration of human placenta, implantation of cold-stored tissues, placenta plasma suspension prepared by homogenization, and the pepsin and/or acid hydrolysate of human placenta, have been utilized for clinical trials.

Since the first report of keratoplasty with a cold-stored corneal graft by Filatow W.P. in 1933, the effectiveness of such tissue therapy has been confirmed in various chronic diseases. The technique developed by Filatow has been expanded from animal and human trials to the plant kingdom and has become widely known as tissue therapy. Not only animal and human tissues, but also plant materials under stressed conditions have been widely used as original sources of so-called "biogenic stimulant" reported by Filatow. In placenta stored at 2-4°C for 4-5 days, nitrogen protein and glucose contents decreased by half, and lactic acid and non-nitrogen protein contents increased by 150%. Among plant sources, the fraction obtained from *Aloe arborescens* kept in cold storage at 4-5°C under the condition of darkness for 12 days, was used as a biogenic stimulant and showed relatively higher levels of malic and tartaric acids than the controls. The effects of *Aloe arborescens* var. *natalensis* (Kidachi aloe), stored under cold and dark conditions for 10 days, on the phagocytosis and NBT-reduction of neutrophils from adult bronchial asthmatic patients, was investigated and found a significant stimulation of anti-inflammatory activity due to the biogenic stimulant of Kidachi aloe, suggesting the hydrolysis of phospholipids releasing arachidonic acids to synthesize prostanoids involving endogeneously present cyclooxygenase in the stored dark and cold conditions in aloe gel. These findings indicate that not putrefaction, but autolysis, glycolysis and lactic acid fermentation proceeded gradually under the condition employed<sup>[6]</sup>.

#### Placenta extract

A dipeptide, cyclo (trans-4-L-hydroxyprolyl-L-serine; JBP485) and  $\delta$ -hydroxylysine isolated from the hydrolysate of human placenta, on the basis of physico-chemical and HPLC examination showed

a proliferation-promoting activity<sup>[7]</sup>. JBP485 exhibited potent antihepatitis activity after oral administration. The increased in bilirubin concentration and activities of liver cytosolic enzymes in serum caused by  $\alpha$ -naphthylisothiocyanate intoxication in rats were significantly countered both after i.v. and oral administration of JBP485. Antihepatitis activity of JBP485 resulted, at least partially, in the direct effect on hepatocytes, because glutamic-oxaloacetic transaminase and lactate dehydrogenase activities in the medium of hepatotoxin-exposed primary cultured hepatocytes were reduced by JBP485. JBP485 is potent anti-hepatitis reagent that is active after oral administration and may be useful for clinical application<sup>[8]</sup>.

#### Aloe vera

*Aloe vera* gel has been widely used for healing of burn wound in second degree and hepatitis with paucity of therapeutic evidence. The quality control of the biologically active principles is necessary for therapeutic applications. By using the patented hyper-dry system after washing out coloured materials with running water, aloe high molecular weight fractions (AHM), were obtained in original and natural form containing less than 10 ppm of barbaloin and were examined for the therapy designed by the implementation of well-controlled clinical trials, and exhibited the efficacy as immunomodulators for viral infection-induced hepatic periportal fibrosis and type 2 diabetic patients, and as wound and ulcer-healing ointment to patients suffering from bed sore and lichen planus. AHM were considered to be a novel low-cost and safe drug candidate of natural origin, indicating a possible therapeutic efficacy in prevention of age-related diseases by slowing aging process<sup>[9]</sup>.

Diet metabolism and immuno-modulatory activity are linked to both to each other, allowing mammals to adapt to diverse changes in their intestinal gut surroundings. The obesity as a primary source of disease brings about metabolic dysfunction followed by inflammatory insulin resistance. The metabolites of aloe polymannose moiety, such as a manno oligosaccharide and short chain fatty acids, synergistically modulated insulin sensitivity on tissues with combination of phenolics, such as aloesin, aloe emodin (a metabolite of barbaloin) and salicylate in *Aloe vera* gel. Insulin levels and insulin activity in the central nerve system, such as hippocampus, are reduced in Alzheimer's disease (AD) and amnestic mild cognitive impairment (MCI). Restoring insulin levels to normal in the brain may provide therapeutic benefit to elderly subjects with AD and amnestic MCI. The effects of aloe polymannose multinutrients complex (APMC) on cognitive and immune function in AD showed the improvements in both clinical and physiological outcomes, though the several limitations of current investigation were noted. APMC may offer an alternative opinion for persons with AD and amnestic MCI<sup>[10]</sup>. Putative prophylaxes of *Aloe vera* for age-related diseases showed that the antioxidative phytochemicals and acemannan (aloe polymannose moiety) could cooperatively contribute to the improvement of health by preventing of age-related diseases through their synergistic systems<sup>[11]</sup>.

Present review was focused on putative prophylaxes updated of placental extract and *Aloe vera* as biogenic stimulants followed by *in vitro*, animal and preliminary clinical studies: defibratide (polydeoxyribonucleotide) from human placenta, metastin and stem cells from human placenta, animal study of placental extract, human study of placental extract; wound healing of *Aloe vera*, bioavailability (drug delivery system) by aloe gel, neuro-stimulation and insulin-sensitivity of *Aloe vera*, bone formation (bone marrow stromal cells) and humoral immune response augmentation of *Aloe vera* gel (acemannan), effect of *Aloe vera* supplementation in subjects with

pre-diabetic/ metabolic syndrome, therapeutic implications, and conclusion and future perspectives.

## DEFIBROTIDE (POLYDEOXYRIBONUCLEOTIDE, PDRN) FROM HUMAN PLACENTA

The PDRN fraction is an extract which forms the active component in a new formulation of the drug placentex (a tissue repair stimulating agent), obtained from human placenta through an original proprietary extraction method. Characterization and quantitation of the active PDRN from human placenta were determined by the authors as follow. From a comparison of the UV, NMR, and IR spectra of this fraction (before and after nuclease treatment) with that of a similar standard (Sigma D1501), it was shown that the active substances in the PDRN fraction mainly consist of a mixture of DNA fragments and were shown to range from 50 to 2000 base pairs by gel electrophoresis. Finally, an HPLC method was described, based on an anion-exchange material capable of determining the amount of PDRN in different batches of the extract, which varied from 80 to 90%<sup>[12]</sup>.

The authors evaluated the effects of PDRN on human cultured osteoblasts, focusing their attention on cell proliferation and alkaline phosphatase activity. PDRN at a concentration of 100 µg/mL induce an increase in osteoblasts growth after 6 days as compared to control (+21%). The addition of DMPX (A2 antagonist) 50 µM and suramine (P2 inhibitor) 10 µM give different results: suramine has no significant effect, while DMPX reduce, even if partially, the PDRN induced cell growth. The alkaline phosphatase activity shows a gradual enhancement starting from day 0 to day 10, even if PDRN treated cells, examined at day 6, present a sensibly lower phosphatase activity when compared to controls. The authors demonstrated that PDRN acts as an osteoblast growth stimulator. Its action is partially due to a stimulation of the purinergic system mediated by A2 purinoreceptors, however the authors can not excluded the involvement of other mechanism like salvage pathway<sup>[13]</sup>.

Defibrotide (DF), a polydeoxyribonucleotide with anti-thrombotic properties, has proven effective in patients with severe hepatic veno-occlusive disease (VOD), a life-threatening complication of high-dose chemo/radiotherapy regimens for stem cell transplantation. To understand the mechanism of its beneficial effect, the authors studied the impact of DF on the expression of tissue factor (TF) and fibrinolytic proteins (PAI-1 and t-PA) on endothelial cells. The *in vitro* response to DF of two types of endothelial cells (EC) of different origins, that is from human umbilical vein endothelial cells (HUVEC) and microvascular endothelial cells (HMEC-1) beds, was evaluated in the presence or absence of a proinflammatory stimulus (ie bacterial endotoxin, LPS). The results show that DF was able to significantly reduce the LPS-induced TF expression by HMEC-1, and less prominently by HUVEC. In addition, DF importantly influenced the fibrinolytic properties of both HMEC-1 and HUVEC. Specifically, it dose-dependently counteracted the LPS-induced increase in PAI-1 levels and decrease in t-PA activity expression. It also significantly incremented t-PA antigen in resting EC. Decreasing the pro-coagulant activity and increasing the fibrinolytic potential of EC favors an anti-coagulant phenotype of the endothelium, which may protect from fibrin deposition and vascular occlusion<sup>[14]</sup>.

Veno-occlusive disease of the liver (VOD) remains a troubling and potentially fatal complication of high-dose chemotherapy and hematopoietic stem cell transportation conditioning regimens. No effective therapy has been available for these patients to date, and the best supportive care measures remain woefully inadequate.

Defibrotide (DF), a polydisperse mixture of all the singlestranded phosphodiester oligodeoxyribonucleotides that can be obtained from the controlled depolymerization of porcine intestinal mucosal genomic DNA, seems to be offer a safe and effective treatment for some patients suffering from severe VOD, a condition for which no accepted standard therapy currently exists. Early clinical studies evaluating the efficacy of DF for the treatment of severe VOD in patients undergoing hematopoietic stem cell transplantation have been very encouraging. Approximately 45% of the patients treated in multiple initial phase II clinical trials achieved a complete response at day +100, demonstrating normalization of serum bilirubin and resolution of the clinical syndrome. However, although multi-institutional, these represented single arm studies. A large, FDA-approved, pivotal, prospective, multi-institutional, global phase III trial of DF vs historical controls (best available therapy) commenced in the first quarter of 2006 and should provide futher validation of DF's efficacy. The drug seems to have few significant side effects, and almost all test subjects who have received this treatment have tolerated it well. Although the mechanism of action remains unclear, the drug exerts minimal systemic anticoagulant effects yet appears to induce numerous antithrombotic and profibrinolytic effects both *in vitro* and *in vivo*. It may function as an adenosine receptor agonist and causes increased concentrations of endogeneous prostaglandins, which modulate thrombomodulin, platelets, and fibrinolysis. It also appears to block lipopolysaccharide- induced tissue factor expression. However, despite the fact the DF is composed of oligonucleotides, its mechanism of action, which at the present time is unclear, is not related to Watson-Crick base pair-dependent downregulation of gene expression but is rather likely a result of its polyanionic nature<sup>[15]</sup>.

The authors compared the effects of topical placental-extract gel and cream of chronic non-healing wound with regard to wound healing and discomfort during dressing change. **Methods:** A sample of 120 patients attending the wound clinic at University Hospital, Varanasi, India, with wound of more than six weeks' duration were enrolled into the study. They were alternately allocated to group A (topical application of placental-extract gel) or group B (placental-extract cream). Wound biopsy was performed, and swab culture and sensitivity were taken. Wound size was measured, and visual analogous scale (VAS) scores for pain and discomfort at dressing change were recorded at weekly follow-up in both groups. Biopsy was repeated after two weeks of treatment and sent for histopathological examination for assessment of of angiogenesis in 25 cases from each group. **Results:** One hundred patients completed the study. More than 50% wound healing was observed after eight weeks in 72% of group A patients and 74% of group B patients ( $p=0.75$ ). Microscopic angiogenesis grading system scores were similar in both groups (not statistically significant,  $p=0.92$ ). The VAS scores for pain and discomfort were lower in group B (statistically significant,  $p<0.02$ ) **Conclusion:** Placental-extract gel and cream are both effective topical agents for chronic non-healing wound. However, there is less pain and discomfort during dressing change with the placental-extract cream, which the author thus recommend for topical application in chronic non-healing wound<sup>[16]</sup>.

Hepatic veno-occlusive disease (VOD) is one of the most important complications of high-dose chemotherapy and stem cell transplantation. VOD is a clinical syndrome characterized by jaundice, hepatic enlargement and fluid retention typically seen by day +30 after transplantation. Severe VOD is complicated by multiorgan failure and a high mortality rate approaching 100%. Defibrotide (DF) is a novel agent with both anti-thrombotic and fibrinolytic properties that has emerged as an effective therapy

for severe VOD. In phase II studies, treatment of severe VOD has resulted in complete responses of 30-60% and survival past day 100 ranging between 32-50%. A phase III, historically controlled study of DF for treatment of severe VOD has recently been completed and results are awaited with interest. In addition, DF may be effective prophylaxis for VOD in high-risk patients. This review focuses on a summary of the pharmacology of DF and the clinical evidence for its use in VOD<sup>[17]</sup>.

Plasmin, a potent and non-specific serine protease, plays a pivotal role in fibrinolysis by virtue of its ability to effectively degrade fibrin clots. Defibrotide increases the activity of plasmin in hydrolyzing its substrate in a dose-dependent and length-dependent manner. Similar concentration-dependent effects of defibrotide were observed when plasmin was generated by tissue plasminogen activator or urokinase activation of plasminogen. In contrast, defibrotide had no direct effect on the activation of plasminogen to plasmin. Defibrotide was also able to enhance the activity of plasmin in degrading fibrin clots formed from fibrinogen, plasminogen, and thrombin. This effect was also concentration-dependent and directly correlated with the enzymatic activity of plasmin. This study therefore demonstrates that defibrotide is capable of enhancing the activity of plasmin and so contributes to its fibrinolytic activity. Taken together, these results support the effect of defibrotide in restoring the fibrinolytic vascular phenotype, in microangiopathic conditions such as veno-occlusive disease<sup>[18]</sup>.

A number of possible strategies for the prevention and/or treatment of hepatic veno-occlusive disease (VOD) in children have been investigated. The most promising agent to date is defibrotide, a novel polydeoxyribonucleotide with fibrinolytic properties but no major bleeding risk. Numerous studies, including phase II/III trials, have shown clinical benefit in pediatric patients with the use of defibrotide treatment and prophylaxis. This review discusses VOD in children and focuses on therapeutic options, including defibrotide, in this patients population<sup>[19]</sup>.

The authors presented a method for treating and structurally improving articulations affected by degenerative joint disease (DJD). The focus of this analysis is on two groups of patients: the first comprised patients over eighty years old, and the second comprised patients aged 45 to 55 years. The first group was a high surgical risk and both had been nonresponders to current conservative therapies. Scholars like Davis, Filatow, and Cerletti have been studying and using the regenerative properties of placenta, amnios and other nonvital tissues since the early 1900s. These pioneering studies have opened a new track for tissue renewal. More recently, the new biological knowledge about extracellular nucleic acids, growth factors (as by-products of trauma response), and heat shock proteins has helped research even further. Building on those experiences, the authors have developed a regenerative gel obtained with distressed, processed blood, polydeoxynucleotides, and a thickening substance. The objective was to stimulate the local innate stem cells with the gel in order induce tissue repair. From 2003 until 2009, the authors treated 948 patients. As mentioned, the first group comprised of 86 ultra-octogenarin patients with severe osteoarthritis of the hip and/or knee, and the second group comprised of 90 younger patients (around 50 years old) affected by the same disease. Treated patients have been clinically and radiologically evaluated with a follow-up of 6 to 48 months. Results show a statistically significant improvement in terms of pain and joint mobility, sometimes coupled with clear improvement in radiological imaging. Follow-up shows encouraging data in terms of clinical stability over time. During the study, the authors encountered virtually no side effects, adverse reactions,

or toxicity. Currently the pharmacological treatment of DJD is palliative, though toxicity and side effects of the drugs remain problematic. Patients who can be operated on conclude their trial with a prosthesis followed by a long rehabilitation period. This study presents a new methodological approach to the treatment of DJD based on tissue regeneration and restoration resulting in a positive clinical resolution<sup>[20]</sup>.

Hepatic veno-occlusive disease is a leading cause of morbidity and mortality after haemopoietic stem-cell transplantation (HSCT). The authors aimed to assess whether defibrotide can reduce the incidence of veno-occlusive disease in this setting. **Methods:** In phase 3 open-label, randomised controlled trial, the authors enrolled patients at 28 European university hospitals or academic medical centres. Eligible patients were younger than 18 years, had undergone myeloablative conditioning before allogenic or autologous HSCT, and had one or more risk factor for veno-occlusive disease based on modified Seattle criteria. The authors centrally assigned eligible participants on the basis of a computer-generated randomisation sequence (1:1), stratified by centre and presence of osteopetrosis, to receive intravenous defibrotide prophylaxis (treatment group) or not (control group). The primary endpoint was incidence of veno-occlusive disease by 30 days after HSCT, adjudicated by a masked, independent review committee, in eligible patients who consented to randomisation (intention-to-treat population), and was assessed with a competing risk approach. Patients in either group who developed veno-occlusive disease received defibrotide for treatment. The authors assessed adverse events to 180 days after HSCT in all patients who received allocated prophylaxis. This trial is registered with ClinicalTrials.gov, number NCT00272948. **Findings:** Between Jan 25, 2006, and Jan 29, 2009, the authors enrolled 356 eligible patients to the intention-to-treat population. Twenty two (12%) of 180 patients randomly allocated to the defibrotide group had veno-occlusive disease by 30 days after HSCT compared with 35 (20%) of 176 controls (risk difference -7.7%, 95% CI -15.3 to -0.1; Z test for competing risk analysis  $p=0.0488$ ; log-rank test  $p=0.0507$ ). One hundred fifty four (87%) of 177 patients in the defibrotide group had adverse event by day 180 compared with 155 (88%) of 176 controls. **Interpretation:** Defibrotide prophylaxis seems to reduce incidence of veno-occlusive disease and is well tolerated. Thus, such prophylaxis could present a useful clinical option for this serious complication of HSCT<sup>[21]</sup>.

## METASTIN AND STEM CELLS FROM HUMAN PLACENTA

The authors aimed to identify the gene(s) responsible for the suppression of metastasis in chromosome 6/melanoma cell hybrids. **Methods:** A modified subtractive hybridization technique was used to compare the expression of messenger RNAs (mRNAs), via an analysis of complementary DNAs (cDNAs), in metastatic cells (C8161 or MelJuSo) and nonmetastatic hybrid clones (neo6/C8161 or neo6/MelJuSo). **Results:** A novel cDNA, designated KiSS-1, formally, metastin encoded by the Kiss 1 gene, was isolated from malignant melanoma cells that had been suppressed for metastatic potential by the introduction of human chromosome 6. Northern blot analyses comparing mRNAs from a panel of human melanoma cells revealed that KiSS-1 mRNA expression occurred only in nonmetastatic melanoma cells. Expression of this mRNA in normal heart, brain, liver, lung, and skeletal muscle was undetectable by northern blot analysis. Weak expression was found in the kidney and pancreas, but the highest expression was observed in the placenta. The KiSS-1 cDNA encodes a predominantly hydrophilic, 164 amino acid protein

with a polyproline-rich domain indicative of an SH3 ligand (binds to the homology 3 domain of the oncoprotein Src) and a putative protein kinase C- $\alpha$  phosphorylation site. Transfection of a full-length KiSS-1 cDNA into C8161 melanoma cells suppressed metastasis in an expression- dependent manner. **Conclusion:** These data strongly suggest that KiSS-1 expression may suppress the metastatic potential of malignant melanoma cells. Implications: KiSS-1 may be a useful marker for distinguishing metastatic melanomas from nonmetastatic melanomas<sup>[22]</sup>.

Metastasis is a major cause of death in cancer patients and involves a multistep process including detachment of cancer cells from a primary cancer, invasion of surrounding tissue, spread through circulation, re-invasion and proliferation in distant organs. KiSS-1 is a human metastasis suppressor gene, that suppresses metastases of human melanomas and breast carcinomas without affecting tumorigenicity. However, its gene product and functional mechanisms have not been elucidated. The authors showed that KiSS-1 encodes a carboxy-terminally amidated peptide with 54 amino-acid residues, which the authors have isolated from human placenta as the endogenous ligand of an orphan G-protein-coupled receptor (hOT7T175) and have named 'metastin'. Metastin inhibits chemotaxis and invasion of hOT7T175-transfected CHO cells *in vitro* and attenuates pulmonary metastasis of hOT7T175-transfected B16-BL6 melanoma *in vivo*. The results suggested possible mechanisms of reaction for KiSS-1 and a potential new therapeutic approach<sup>[23]</sup>.

To investigate the possibility that metastin is an endocrine peptide, the authors determine the immunoreactive (ir)-metastin concentration in human plasma using the new developed, sensitive, and specific two-site enzyme immunoassay. The plasma concentrations of ir-metastin in males and females were  $1.30 \pm 0.14$  ( $n=12$ ) and  $1.31 \pm 0.37$  fmol/ml ( $n=10$ ), respectively. As metastin is known to be abundant in human placenta, the ir-metastin concentration in the maternal plasma was then determined. The ir-metastin concentrations were  $1.230 \pm 346$  fmol/mL ( $n=11$ ) in the first trimester,  $4590 \pm 555$  ( $n=16$ ) in the second trimester, and  $9.590 \pm 1640$  ( $n=12$ ) in the third trimester. On d 5 after delivery, the ir-metastin concentration returned to nearly the nonpregnant level ( $7.63 \pm 1.33$  fmol/mL;  $n=10$ ), suggesting that ir-metastin increases in pregnancy and is derived mainly from the placenta. The plasma from both nonpregnant and pregnant women showed a single ir-metastin peak at the same retention time as authentic metastin on reverse phase HPLC analyses, indicating that the major portion of the circulating metastin, as determined by the two-site enzyme assay, represents endogenous metastin. Histochemical studies of human placenta localized metastin mRNA and immunoreactivity to the syncytiotrophoblasts. The present study provides evidence for metastin as a novel plasma-derived hormone in humans<sup>[24]</sup>.

Kiss-1 is a human metastasis suppressor gene that inhibits metastasis of human melanomas and breast carcinomas without affecting tumorigenicity. Kiss-1 encodes a carboxy-terminally amidated peptide with 54 amino-acid residues. The peptide was isolated from human placenta as the endogenous ligand of an orphan G-protein-coupled receptor and termed 'metastin'. The literature reports metastin related to human carcinoma, such as melanoma, thyroid cancer, esophageal squamous cell carcinoma (ESCC), hepatocellular carcinoma, pancreatic carcinoma, as well as breast, ovarian, bladder and kidney cancer. These malignancies are difficult to treat and even in early-stage cancer, a number of patients develop metastasis shortly after surgery. Studies have suggested that metastin inhibits tumor invasion or migration through focal adhesion kinase, paxillin, MAP kinase or Rho A. Additionally, metastin may be a

biomarker in ESCC, pancreatic carcinoma and bladder cancer. Metastin has potential as a suitable biomarker in the identification of tumors with high metastatic potential and as a novel effective treatment modality for patients with metastasis<sup>[25]</sup>.

Alzheimer's disease (AD) onset is associated with changes in hypothalamic-pituitary- gonadal (HPG) function. The 54 amino acid kisspeptin (KP) peptide regulates the HPG axis and alters antioxidant enzyme expression. The Alzheimer's amyloid- $\beta$  (A $\beta$ ) is neurotoxic, and this action can be prevented by the antioxidant enzyme catalase. The authors examined the effect of KP peptides on the neurotoxicity A $\beta$ , prion protein (PrP), and amylin (islet amyloid polypeptide: IAPP) peptides. The A $\beta$ , PrP, and IAPP peptides stimulated the release of KP and KP 45-54. The KP peptides inhibited the neurotoxicity of A $\beta$ , PrP, and IAPP peptides, via an action that could not be blocked by kisspeptin- receptor (GPR-54) or neuropeptide FF (NPFF) receptor antagonists. Knockdown of Kiss-1 gene, which encodes the KP peptides, in human neuronal SH-SY5Y cells with siRNA enhanced the toxicity of amyloid peptides, while Kiss-1 overexpression was neuroprotective. A comparison of the catalase and KP sequences identified a similarity between KP residues 42-51 and the region of catalase that binds A $\beta$ . The KP peptides containing residues 45-50 (YNWNSF) bound A $\beta$ , PrP, and IAPP, inhibited Congo red binding, and were neuroprotective. These results suggest that KP peptides are neuroprotective against A $\beta$ , IAPP, and PrP peptides via a receptor independent action involving direct binding to the amyloid peptides<sup>[26]</sup>.

The pons region of the Alzheimer's disease (AD) brain is one of the last to show amyloid- $\beta$  (A $\beta$ ) deposits and has been suggested to contain neuroprotective compounds. Kisspeptin (KP) is a hormone that activates the hypothalamic-pituitary-gonadal axis and has been suggested to be neuroprotective against A $\beta$  toxicity. The localization of KP, plus the established endogenous neuroprotective compounds corticotropin releasing hormone (CRH) and catalase, in tissue sections from the pons region of a male AD subject has been determined in relation to A $\beta$  deposits. Results showed A $\beta$  deposits also stained with KP, CRH, and catalase antibodies. At high magnification the staining of deposits was either KP or catalase positive, and there was only a limited area of the deposits with KP-catalase colocalization. The CRH does not bind A $\beta$ , whilst both KP and catalase can bind A $\beta$ , suggesting that colocalization in A $\beta$  deposits is not restricted to compounds that directly bind A $\beta$ . The neuroprotective actions of KP, CRH, and catalase were confirmed *in vitro*, and fibrillar A $\beta$  preparations were shown to stimulate the release of KP *in vitro*. In conclusion, neuroprotective KP, CRH, and catalase all colocalized with A $\beta$  plaque-like deposits in the pons region from a male AD subject<sup>[27]</sup>.

The authors investigated the neuroprotective effects of human placental extract (HPE) and the effects of HPE on recovery of cognitive and behavioral function on hypoxic-ischemic brain injury in the newborn rat. The right common carotid arteries of 7-day-old rats were coagulated, and rats were then exposed to 8% oxygen. Immediately before and again at three times after the hypoxia-ischemia (pre-treatment group), and immediately after and three times again after hypoxia-ischemia (post-treatment group), the rats were intra-peritoneally injected with HPE (0.1, 0.25, or 0.5 mL/10g/ dose). No-treatment rats received saline only. On postnatal day 12, brains were removed and gross morphological damage was evaluated. To quantify the severity of brain injury, bilateral cross-sectional areas of the anterior commissural and posterior hippocampal levels were analyzed with NIH Image. Assessments of the open field activity levels at 2, 4, 6 and 8 week and, the Morris water maze

test at 8 weeks after hypoxia-ischemia were carried out according to standard methods. HPE pre-treatment decreased the incidence of liquefactive cerebral infarction, at an optimally neuroprotective dose of 0.5 mL/10 g/dose ( $p < 0.05$ ). In the Morris water maze test, the group injected with HPE at 0.5 mL/10g/dose concentration showed shorter escape latencies than the no-treatment group ( $p < 0.05$ ). These findings support a protective effect of the HPE treatment on neuronal integrity and cognitive function following hypoxic-ischemic brain injury. Injected at an appropriate dose prior to exposure, HPE may significantly reduce or prevent hypoxic-ischemic injury in the immature brain<sup>[28]</sup>.

The authors investigated the therapeutic effects of PDA-001, a culture expanded placenta-derived adherent cell, in the rat neuritis model. Pain is induced in the model by applying carrageenan to the sciatic nerve trunk, causing perineural inflammation of the sciatic nerve. PDA-001, at doses ranging from  $0.4 \times 10^6$  to  $4 \times 10^6$  cells/animal, or vehicle control was intravenously administered to assess the biological activity of the cells. A dose-dependent effect of PDA-001 on pain relief was demonstrated. PDA-001 at doses of  $1 \times 10^6$  and  $4 \times 10^6$ , but not  $0.4 \times 10^6$ , reduced mechanical hyperalgesia within 24 h following treatment and through day 8 after induction of neuritis. The mechanism underlying PDA-001 mediated reduction of neuroinflammatory pain was also explored. *Ex vivo* tissue analyses demonstrated that PDA-001 suppressed homing, maturation and differentiation of dendritic cells, thus inhibiting T-cell priming and activation in draining lymph nodes. PDA-001 also reduced interferon  $\gamma$  and IL17 in draining lymph nodes and in the ipsilateral sciatic nerve, and increased the levels of IL10 in draining lymph nodes and plasma, pointing to T-cell modulation as a possible mechanism mediating the observed anti-hyperalgesic effects. Furthermore, in the ipsilateral sciatic nerve, significantly less leukocyte infiltration was observed in PDA-001-treated animals. The results suggest that PDA-001 may provide a novel therapeutic approach in the management of inflammatory neuropathic pain and similar conditions<sup>[29]</sup>.

Human placenta has emerged as a valuable source of transplantable cells of mesenchymal and hematopoietic origin for multiple cytotherapeutic purposes, including enhanced engraftment of hematopoietic stem cells, modulation of inflammation, bone repair, and cancer. Placenta-derived adherent cells (PDACs) are mesenchymal-like stem cells isolated from postpartum human placenta. Multiple myeloma is closely associated with induction of bone disease and large lytic lesions, which are often not repaired and are usually the sites of relapses. The authors evaluated the antimyeloma therapeutic potential, *in vivo* survival, and trafficking of PDACs in the severe combined immunodeficiency (SCID)-rab model of medullary myeloma-associated bone loss. Intrabone injection of PDACs into non-myelomatous and myelomatous implanted bone in SCID-rab mice promoted bone formation by stimulating endogenous osteoblastogenesis, and most PDACs disappeared from bone within 4 weeks. PDACs inhibitory effects on myeloma bone disease and tumor growth were dose-dependent and comparable with those of fetal human mesenchymal stem cells (MSCs). Intrabone, but not subcutaneous, engraftment of PDACs inhibited bone disease and tumor growth in SCID-rab mice. Intratumor injection of PDACs had no effect on subcutaneous growth of myeloma cells. A small number of intravenously injected PDACs trafficked into myelomatous bone. Myeloma cell growth rate *in vitro* was lower in coculture with PDACs than with MSCs from human fetal bone or myeloma patients. PDACs also promoted apoptosis in osteoclast precursors and inhibited their differentiation. This study suggests that altering the bone marrow microenvironment with PDAC cytotherapy attenuates

growth of myeloma and that PDAC cytotherapy is a promising therapeutic approach for myeloma osteolysis<sup>[30]</sup>.

The clinical utility of cellular therapies is being investigated in a broad range of therapeutic areas. The phase 1 study represents the first exploration of PDA001, a preparation of cells cultured from human placental tissue, in subjects with Crohn's disease. Methods: Twelve subjects with active, moderate-to-severe Crohn's disease unresponsive to previous therapy were given 2 intravenous infusions of PDA001 1 week apart, monitored weekly for 5 weeks, and assessed at 6 months, 1 year, and 2 years after infusion. Six subjects received 2 infusions of  $2 \times 10^8$  cells (low dose), and 6 subjects received 2 infusions of  $8 \times 10^8$  cells (high dose). Results: Mean baseline Crohn's Disease Activity Index in the low-dose and high-dose groups was 305 and 365, respectively, and mean C-reactive protein was 8 mg/L and 49 mg/L, respectively. All subjects in the low-dose group achieved a clinical response (a Crohn's Disease Activity Index decrease of  $\geq 70$  points versus baseline), and 3 achieved remission (a Crohn's Disease Activity Index decrease of  $\geq 100$  to  $< 150$  points). Two subjects in the high-dose group achieved response, and none met remission criteria. Most adverse events were mild to moderate in severity and included headache, nausea, fever, and infusion site reactions. **Conclusion:** PDA001 infusions appear safe and well-tolerated in subjects with treatment-resistant Crohn's disease. Response was seen in all subjects in the low-dose group. The high-dose group, with a higher baseline disease activity, had only 2 responders, suggesting a more treatment-resistant population. A phase 2 study in this patients population is ongoing<sup>[31]</sup>.

Human placenta-derived adherent (PDA001) cells are mesenchymal-like stem cells isolated from postpartum placenta. The authors tested whether intravenously infused PDA001 improves neurological functional recovery after stroke in rats. In addition, potential mechanisms underlying the PDA001-induced neuroprotective effect were investigated. Young adult rats (2-3 months) were subjected to 2 h of middle cerebral artery occlusion (MCAo) and treated with PDA001 ( $4 \times 10^6$ ) or vehicle controls [dextran vehicle or phosphate buffer saline (PBS)] via intravenous administration initiated at 4 h after MCAo. A battery of functional tests and measurements of lesion volume and apoptotic cells were performed. Immunostaining and ELISA assays for vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) and brain derived neurotrophic factor (BDNF) were performed in the ischemic brain to test the potential mechanisms underlying the neuroprotective effects of PDA001 cell treatment of stroke. PDA001 cell treatment at 4 h post stroke significantly improved functional outcome and significantly decreased lesion volume, TUNEL assay, and cleaved caspase 3-positive cell number in the ischemic brain, compared to MCAo-vehicle and MCAo-PBS control. Treatment of stroke with PDA001 cells also significantly increased HGF and VEGF expression in the ischemic border zone (IBZ) compared to control. Using ELISA assays, treatment of stroke with PDA001 cells significantly increased VEGF, HGF, and BDNF levels in the ischemic brain compared to control. Conclusion: When administered intravenously at 4 h after MCAo, PDA001 cells promoted neuroprotective effects. These effects induced by PDA001 cell treatment may be related to the increase of VEGF, HGF, and BDNF expression, and a decrease of apoptosis. PDA001 cells may provide a viable cell source to treat stroke<sup>[32]</sup>.

The authors investigated the efficacy of PDA001 treatment in a rat model of transient middle cerebral artery occlusion (MCAo) in young adult (2-3 month old) and older rats (10-12 months old). **Methods:** To evaluate efficacy and determine the optimal number of



transplanted cells, young adult Wistar rats were subjected to MCAo and treated 1 day post MCAo with  $1 \times 10^6$ ,  $4 \times 10^6$  or  $8 \times 10^6$  PDA001 cells (i.v.), vehicle or cell control,  $4 \times 10^6$  or  $8 \times 10^6$  PDA001 cells were also tested in older rats after MCAo. Treatment response was evaluated using a battery of functional outcome tests, consisting of adhesive-removal test, modified Neurological Severity Score (mNSS) and foot-fault test. Young adult rats were sacrificed 56 days after MCAo, older rats were sacrificed 29 days after MCAo, and lesion volumes were measured using H&E. Immunohistochemical stainings for bromodeoxyuridine (BrdU) and von Willebrand Factor (vWF), and synaptophysin were performed. **Results:** In young adult rats, treatment with  $4 \times 10^6$  PDA001 cells significantly improved functional outcome after stroke ( $p < 0.05$ ). In older rats, significant functional improvement was observed with PDA001 cell therapy in both of the  $4 \times 10^6$  and  $8 \times 10^6$  treatment groups. Functional benefits in young adult and older rats were associated with significant increases in the number of BrdU immunoreactive endothelial cells, vascular density and perimeter in the ischemic brain, as well as significantly increased synaptophysin expression in the ischemic border zone ( $p < 0.05$ ). **Conclusion:** PDA001 treatment significantly improved functional outcome after stroke in both young and adult rats. The neurorestorative effects induced by PDA001 treatment may be related to increased vascular density and synaptic plasticity<sup>[33]</sup>.

## ANIMAL STUDY OF PLACENTAL EXTRACT

The authors investigated whether human placental extract (HP) treatment in an experimental sciatic nerve injury animal model produces growth-promoting effects on regenerating peripheral nerve fibers after injury. **Methods:** After HP was injected into a sciatic nerve injury site, changes in protein levels were analyzed in the regenerating nerve area by Western blotting and immunofluorescence staining analyses. For quantitative assessment of axonal regeneration, a retrograde tracing technique was used to identify the neuronal cell bodies corresponding to regenerating axons, and the extent of neurite outgrowth in cultured dorsal root ganglia (DRG) sensory neurons prepared from animals that had experienced a sciatic nerve crush injury 7 d before neuron collection was analyzed. **Results:** Induction levels of axonal growth-associated protein (GAP-43) in the injured sciatic nerves were elevated by HP treatment. HP treatment also upregulated cell division cycle 2 (Cdc-2) protein levels in the distal stump of the injured sciatic nerve. Induced Cdc-2 protein was detected in Schwann cells, suggesting that Cdc-2 kinase activity may be involved in the growth-promoting activity of regenerating axons via Schwann cell proliferation. Cell body measurement by retrograde tracing indicated that HP treatment produced significant increases in regenerating motor axons. Finally, HP treatment of cultured DRG sensory neurons significantly increased neurite arborization and elongation. **Conclusion:** HP promotes the regeneration of injured sciatic axons by upregulating the synthesis of regeneration-related protein factors such as GAP-43 and Cdc-2<sup>[34]</sup>.

The authors investigated preventive and therapeutic effects of human placental extract (PE) in contact hypersensitivity (CHS), a mouse model of allergic contact dermatitis. Administration of PE prior to the sensitization of allergic antigen (Ag) significantly inhibited the severity of CHS induced by Ag challenge. This effect was associated with reduced numbers of CD4<sup>+</sup> T cells in peripheral blood, decrease of tissue-infiltrating lymphocytes, and preferential production of Th2-type cytokines in Ag-challenged sites. In addition, CHS caused by repetitive challenges of allergic Ag was also prevented and treated by administration of PE. Finally, administration

of cyclo-trans-4-L-hydroxy- prolyl-L-serine (JBP485), a dipeptide derived from PE, also alleviated CHS, suggesting its potential role in the effects of PE in CHS. Taken together, these findings demonstrated experimental evidence supporting immunoregulatory effects of PE in allergic skin diseases and elucidated its potential mechanisms<sup>[35]</sup>.

The authors aimed to determine whether protective effects of JBP485 on biliary obstruction induced by  $\alpha$ -naphthylisothiocyanate (ANIT) are mediated by the organic anion transporters Oat1, Oat3 and the multidrug resistance-associated protein (Mrp2). The ANIT-induced increases in bilirubin (BIL), alanine aminotransferase (ALT) and aspartate transaminase (AST) in rat serum were inhibited significantly by oral administration of JBP485. The plasma concentration of JBP485 which is the substrate of Oat1 and Oat3 determined by LC-MS/MS was markedly increased after intravenous administration in ANIT-treated rats, whereas cumulative urinary excretion of JBP485 *in vivo* and the uptake of JBP485 in kidney slices were decreased remarkably. RT-PCR and Western blot showed the decreased expression of Oat1 and Oat3, increased expression of Mrp2 in ANIT-induced rats, meanwhile, the expression levels of Mrp2 and Oat1 were up-regulated after administration of JBP485. The up-regulation of Mrp2 and Oat1 was associated with a concomitant increase in urinary BIL after treatment with JBP485 in ANIT-treated rats. The mechanism for JBP485 to restore liver function might be related to improvement of the expression and function for Oat1 and Mrp2 as well as facilitation of urinary excretion for hepatotoxic substance<sup>[36]</sup>.

The authors investigated whether the targets of drug-drug interactions (DDIs) between JBP485 and acyclovir (HSV/VZV infection drug) are OAT1 and OAT3 in kidney. Plasma concentration and accumulative urinary excretion of acyclovir *in vivo*, uptake of acyclovir in kidney slices and uptake of acyclovir in human (h)-OAT1/OAT3-human embryonic kidney (HEK) 293 cells *in vitro* were performed to examine the effect of JBP485 on urinary excretion of acyclovir. The plasma concentration of acyclovir was increased markedly and accumulative urinary excretion and renal clearance of acyclovir were decreased significantly after intravenous administration of acyclovir in combination with JBP485. JBP485 (a substrate for OAT1 and OAT3), p-aminohippurate (a substrate for OAT1) and benzylpenicillin (a substrate for OAT3) could decrease the uptake of acyclovir in kidney slices and in hOAT1/hOAT3-HEK293 cells. These results suggested that JBP485 inhibits the renal excretion of acyclovir by inhibiting renal transporters OAT1 and OAT3 *in vivo* and *in vitro*, suggesting that the possibility of DDIs between dipeptide (JBP485) and acyclovir<sup>[37]</sup>.

The authors aimed to elucidate whether entecavir (HBV prevention drug) was a substrate of OAT1, OAT3, OCT (organic cation transporter), and PEPT1 and to investigate the targets of drug-drug interactions between entecavir and JBP485. Plasma and urine concentrations of entecavir following intravenous and oral administration *in vivo*, uptake of entecavir in kidney slices and transfected cells *in vitro*, were determined by LC-MS/MS. Following intravenous co-administration of entecavir and JBP485 in rats, entecavir AUC increased 1.93-fold,  $t_{1/2\beta}$  was prolonged 2.08-fold, CLP decreased 49%, CLR decreased 73%, and accumulated urinary excretion decreased 54%. However, following oral co-administration, the entecavir  $T_{max}$  and  $C_{max}$  were not affected; the degree of change in other pharmacokinetic parameters (AUC,  $t_{1/2\beta}$ , CLP, and accumulated urinary excretion) was similar to that of intravenous administration. The uptake of entecavir was nearly identical in hPEPT1- as in vector-HELA cells. In rat kidney slices, uptake of entecavir was markedly inhibited by p-aminohippurate,

benzylpenicillin, JBP485, and tetraethyl ammonium. In hOAT1- and hOAT3-HEK293 cells, uptake of entecavir was significantly higher compared to vector-HEK293 cells and was markedly inhibited by p-aminohippurate, benzylpenicillin, and JBP485. Km and Vmax values of entecavir were 250  $\mu$ M and 0.83 nmol/mg protein/30s (OAT1), and 23  $\mu$ M and 1.1 nmol/mg protein/30s (OAT3), respectively. Entecavir is the substrate of OAT1, OAT3, and OCT. Moreover, OAT1 and OAT3 are the targets of DDIs between entecavir and JBP485<sup>[38]</sup>.

The authors examined the effect of placental extract on the memory impairment and neuromorphological change in ovariectomy(OVX)/stress-subjected mice. Female Slc: ICR strain mice were randomly divided into four groups: vehicle-treated OVX, porcine placental extract (120 and 2.160 mg/kg)-treated OVX, and sham-operated control groups. Two weeks after surgical operation, OVX mice underwent restraint stress for 21 days (6 h/day), and all animals were then subjected to a contextual fear conditioning test followed by morphological examination by Nissl staining. Placental extract was orally administered once daily until the behavioral analysis was carried out. Chronic treatment with both doses of placental extract improved the OVX/stress-induced fear memory impairment and Nissl-positive cell loss of the hippocampal CA3 region, although it did not affect the loss of bone mineral density and increase in body weight after OVX. These results have important implications for the neuroprotective and cognitive- enhancing effects of placental extract in postmenopausal women<sup>[39]</sup>.

The authors examined the protective effects of porcine placental extract (PE) against radiation injury. C57BL/6 mice were exposed to 1Gy of r-radiation every day for 5 days, and PE (1 mg/day) was administered orally soon after each exposure. At 2 days after the last irradiation, mice were euthanized to examine the numbers, colony-forming capacity, and DNA damage of stem/progenitor cells in the peripheral blood and bone marrow. To understand the related mechanisms, the authors also measured the levels of intracellular and mitochondrial reactive oxygen species (ROS), and 8-OHdG in the plasma and urine, and IL-6 and TNF- $\alpha$  in the plasma. Compared with the placebo treatment, oral administration of PE significantly increased the number and colony-forming capacity, but decreased the DNA damage of bone marrow stem/progenitor cells. However, neither the levels of intracellular and mitochondrial ROS in bone marrow cells, nor the levels of 8-OHdG in the urine and plasma significantly differed between groups. Interestingly, in comparison with the placebo treatment, PE significantly decreased the levels of the inflammatory cytokines IL-6 and TNF- $\alpha$  in the plasma. PE significantly attenuated the acute radiation injury to bone marrow-derived stem/progenitor cells, and this protection is likely to be related to anti-inflammatory activity of the PE<sup>[40]</sup>.

The authors investigated to explore anticonvulsant property of human placental extract (HPE) in pentylenetetrazole (PTZ) induced convulsions in albino mice. Methods: Effects of HPE, 1.0, 1.2 and 1.4 mL/100 g body weight) as test drug, sodium valproate (150 mg/kg body weight) as standard and distilled water as control were studied in PTZ induced convulsions in albino mouse model. Failure to observe even a single episode of tonic spasm for 5 sec. duration for 1 h was taken as index of anticonvulsant activity. Onset, duration, complete recovery from convulsion and percent protection was calculated and statistical analysis was carried out using student 't' test. Results: Pretreatment with HPE administered in the dose of 1.0 mL/100 g body weight provided 33.33% and in the doses of 1.2 and 1.4 mL/10 g body weight and sodium valproate provided 100 % protection from convulsions induced by PTZ in albino mice.

Conclusion: HPE has shown promising anticonvulsant effect on PTZ induced mouse model<sup>[41]</sup>.

## HUMAN STUDY OF PLACENTAL EXTRACT

The effect of polydeoxyribonucleotide (PDRN) eye drops vs placebo on corneal epithelial healing after photorefractive keratectomy (PRK) for correction of myopic and myopic-astigmatic defects was evaluated in a randomized, double-blind clinical trial. Primary endpoint for efficacy was the evolution of corneal re-epithelialization. Secondary endpoint was the evaluation of PDRN eye drops tolerability. **Methods:** Sixty eyes were enrolled in the study, randomly allocated into standard therapy plus placebo eye drop (30 eyes), or standard therapy plus PDRN eye drops (30 eyes). Checks were carried out preoperatively and at days 1, 2, 3, and 7 of the follow-up. Six eyes dropped out (four in PDRN group, two in placebo group) for reasons unrelated to the study. **Results:** On day 2, the dis-epithelialized area was 8.4 mm<sup>2</sup>  $\pm$ 9.2 (mean $\pm$ SD) in controls and 6.0 mm<sup>2</sup>  $\pm$ 6.8 in PDRN group. On day 3 a complete corneal re-epithelialization was found in 20 out of 26 (77%) eyes of PDRN group and in 17 out of 28 (61%) eyes of placebo group ( $p$ <0.05 in percentage terms). On day 7 of follow-up, all eyes appeared to be completely re-epithelialized. The mean score of corneal evaluation on day 3 was 2.9 in PDRN group and 3.75 in control group ( $p$ <0.05 between groups). No adverse events occurred during the study. **Conclusion:** The data of the study have shown that after PRK, PDRN stimulates corneal epithelium regeneration. PDRN eye drops administration four times a day is well tolerated by patients during the re-epithelialization stage. A much larger clinical study should be performed in order to prove the results obtained in this pilot study<sup>[42]</sup>.

The authors aimed to assess the efficacy and safety of human placenta extract (HPE) in the relief of climacteric symptoms. **Methods:** A prospective, randomized, double-blind, placebo-controlled trial was performed on 108 women with menopausal symptoms. HPE or placebo was administered to the women for 4 weeks. Climacteric symptoms were assessed with the Kupperman Index (KMI). **Results:** Both groups showed a significant reduction in the KMI score at the end of treatment. However, the decrease in the KMI score was significantly greater in the product group than in the placebo group (-12.30 $\pm$ 10.44 vs -7.15 $\pm$ 9.11,  $p$ =0.012) after 4 weeks of treatment. The level of lipid profiles and liver function tests demonstrated no significant changes before and after treatment in both groups. **Conclusion:** HPE reduced climacteric symptoms more than the placebo. The safety evaluation showed a good safety and tolerability profile in the HPE group. The results of the present study suggest that HPE can be an alternative therapy in women with menopausal symptoms<sup>[43]</sup>.

The authors studied the effect and mechanism of human placental extract (HPE) on wound healing. Ten mice (imprinting control region mice, 5 week old males, 30 g) were divided into an experimental group (EG) and a control group (CG). An 8-mm diameter single full-thickness skin defect was made on the back by skin punch biopsy. At least 2.0 $\times$ 10<sup>-3</sup> mL/30 g HPE was injected into the boundaries of the wound. Wound size measurements were taken by digital image every 3 days over 2 weeks. Hematoxylin and eosin (H and E), transforming growth factor- $\beta$  (TGF- $\beta$ ), vascular endothelial growth factor (VEGF), and CD31+ immunohistochemical stains were performed on the 6<sup>th</sup> and 14<sup>th</sup> day. EG showed acceleration in the decrease of wound size compared with CG from the third day to ninth day. TGF- $\beta$  on the 6<sup>th</sup> day showed a statistically significant increase in the EG. VEGF on the 14<sup>th</sup> day showed a statistically significant increase in EG. CD31+



was increased in EG as wound healing progressed, but this increase was not statistically significant. The total number of vessels increased in EG, but this was not statistically significant. The authors concluded that administrating HPE directly to a wound margin promoted wound healing. The mechanism appears to be related to an increase in TGF- $\beta$  in the early phase of wound healing and VEGF in the late phase<sup>[44]</sup>.

The aim of the study was to examine the effects of human placental extract (HPE) on menopausal symptoms, fatigue, and risk factors for cardiovascular disease in middle-aged Korean women in a randomized controlled trial. Korean women, aged 40 to 64 years, with menopausal symptoms and fatigue were recruited as participants. The women were randomly assigned to a placebo group (PG) or an HPE group. The HPE group received subcutaneous injection of HPE in abdomen for 8 weeks, whereas the PG received normal saline. Then, the Menopause Rating Scale (MRS), and Fatigue Severity Scale (FSS), and Visual Analog Scale (VAS) were administered, and risk factors for cardiovascular disease were assessed. The MRS total baseline score was not different between the two groups; however, the score of the HPE group decreased significantly at 8 weeks compared with that of the PG ( $p=0.033$ ). FSS and VAS scores of the PG did not change, whereas the scores of the HPE group decreased significantly during the study period (FSS,  $p=0.002$ ; VAS,  $p=0.001$ ). The baseline 17 $\beta$ -estradiol level was not significantly different between the two groups, but the 17 $\beta$ -estradiol level of the HPE group was significantly increased at 8 weeks compared with that of the PG ( $p=0.031$ ). No changes in risk factors for cardiovascular disease were observed in either groups. Menopausal symptoms and fatigue in middle-aged Korean women improved after 8 weeks of HPE treatment, whereas risk factors for cardiovascular disease did not change during the study period<sup>[45]</sup>.

Combination of placentex injection (an aqueous extract of human placenta) with antimicrobial therapy is better option for treating pelvic inflammatory disease (PID) than only antimicrobial therapy; which also gives more sustained effect and lesser recurrences. Improvements in symptoms continue despite stopping therapy. Addition of placentex leads to marked improvement in dyspareunia, less fornix tenderness and better uterine mobility suggesting better effect on adrenal tissues and parametrium. As placentex decreases adnexal inflammation to significant level in comparison to antimicrobials alone, it can be a good option-specifically to reduce the risk of tubal damage, infertility and formation of adnexal mass. In a study of 100 cases of PID, 50 in group I and II each; 42 in group I and 32 group II completed the study. Marked reduction in various symptoms was observed in study group I (27-59%) whereas in group II where only antimicrobial was given, reduction was found in range of 12-48%. Thus overall, group I had better as well as sustained effect of therapy on relieving dyspareunia, fornix tenderness and uterine restricted mobility which was statistically significant. In other symptoms and signs, there was marginal efficacy except in cervical erosion where no improvement was observed<sup>[46]</sup>.

The authors investigated the effects of human placental extract (HPE) on health status in elderly Koreans. **Design:** Randomized, single-blind, and case-control study. **Setting and participants:** Thirty-nine community-dwelling healthy Koreans  $\geq 65$  years of age. **Intervention:** The participants were randomly categorized into a placebo group (PG:  $n=17$ ) and HPE group ( $n=22$ ). The HPE group received abdominal subcutaneous injection of HPE for 8 weeks. The PG was injected with normal saline. **Measurements:** The degree of health status was surveyed by the Korean health status measure for the elderly (KoHSME V1.0) at baseline and the end of the study.

**Results:** In the HPE group, the scores of physical function, sexual life, and general health perception at the end of the study period were significantly improved from baseline ( $p=0.007$ , 0.020, and 0.005, respectively), while the health status of the PG remained unchanged during the study period. There was a significant difference over the study period between the two groups in the mean change of the physical function score ( $p=0.036$ ). **Conclusion:** A HPE injection regimen can improve the health status in elderly Koreans<sup>[47]</sup>.

The authors conducted to assess the efficacy and safety of the human placental extract solution, which has been known to have a fatigue recovery effect. **Methods:** A total of 315 subjects were randomly assigned to three groups: group 1 (with Unicenta solution administration), group 2 (with exclusively human placental extract administration, excluding other ingredients from the Unicenta solution), and the placebo group. Subsequently, solutions were administered for four weeks. **Results:** The fatigue recovery rate was 71.00% in group 1, 71.72% in group 2, and 44.2% in the placebo group, which show statistically significant differences between the group 1 and the placebo group ( $p=0.0002$ ), and between group 2 and the placebo group ( $p=0.0001$ ). **Conclusion:** The human placental extract solution was effective in the improvement of fatigue<sup>[48]</sup>.

## WOUND HEALING OF ALOE VERA

The authors performed complete chemical analysis at first. Inorganic substances (eg, sodium, potassium, chloride, calcium, and inorganic phosphorous) along with organic compounds (eg, glucose, protein, cholesterol, triglycerides and salicylic acid) were found to be present. Trace metal analysis revealed that Mg and Zn were also present. The bactericidal effects of the extract were also examined. Concentrations as low as 60% were found to be bactericidal against nine of the 12 species of organisms tested.

These were *Citro bacter* sp., *Serratia marcescens*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyrogenes*, *S. agalactiae*, and *Candida albicans*. The remaining three species, *Escherichia coli*, *Streptococcus faecalis*, and *Bacillus subtilis*, all exhibited some resistance to the 60% concentration. However, all of these were susceptible to concentrations of 80% and 90%. A cream base containing 70% oil in *Aloe vera* extract was found to be most effective in preserving the dermal microcirculation after thermal injury. This compound was demonstrated to inhibit some of the products of arachidonic acid metabolism such as thromboxane B, and to limit the production of prostaglandin F/X, thus preventing progressive dermal ischemia. These experimental data clearly show that the effects elicited by the *Aloe vera* extract are truly beneficial in the burn wound<sup>[49]</sup>.

The authors examined antioxidant components in *Aloe vera* for lipid peroxidation using rat liver microsomal and mitochondrial enzymes. Among the aloesin derivatives examined, isorabaichromone showed a potent antioxidative activity. The DPPH radical and superoxide anion scavenging activities were determined. As one of the most potent components, isorabaichromone together with feruloylaloetin and p-coumaroylaloetin showed potent DPPH radical and superoxide anion scavenging activities. Electron spin resonance using the spin trapping method suggested that the potent superoxide anion scavenging activity of isorabaichromone may have due to its caffeoyl group. As *Aloe vera* has long been used to promote wound healing, the inhibitory effects of aloesin derivatives for cyclooxygenase-2 and thromboxane A<sub>2</sub> synthase were examined and the participation of p-coumaroyl and feruloyl ester groups in the

aloenin skeleton was demonstrated. These findings may explain, at least in part, the wound healing effects of *Aloe vera*<sup>[50]</sup>.

An active glycoprotein fraction containing 58% protein, was isolated from *Aloe vera* gel by precipitation with 55% ammonium sulfate followed by gel permeation using DEAE-Sephacel A-25, Sephrose 6B and Sephadex G-50 columns in a yield of  $3 \times 10^{-3}\%$ . The glycoprotein fraction showed a single band corresponding to a subunit of verectin with molecular weight of 29 kD, at the same position when stained with both Coomassie brilliant blue and periodic acid-Schiff reagents on 18% SDS-PAGE. The molecular weight (14 kDa) was confirmed by Sephadex G-50 column chromatography. The glycoprotein fraction showed a radical scavenging activity against superoxide anion generated by the xanthin-xanthine oxidase system as well as inhibition of cyclooxygenase-2 and reduction of thromboxane A2 synthase level *in vitro*<sup>[51]</sup>.

The authors demonstrated the beneficial effects of *Aloe vera* gel on the platelet aggregation in cerebral microvessels of mice. Materials and method: Male mice were injected with saline (control) or *Aloe vera* gel juice (0.2 mL/30g b.w.) one hour before the experiment. Animals were anesthetized and trachea was intubated. Craniotomy was performed and a window was opened on the left side of the skull. Layer of dura was removed. Brain surface microvessels were exposed and animal was placed on the microscope stage. Microscope was connected to a monitor and VCR to record all events. Exposed brain surface was continuously irrigated with artificial cerebrospinal fluid solution. After the body temperature was maintained at 37°C, sodium fluorescein (2%, 0.1 mL/10 g) was injected i.v. through tail injection. After 30 seconds, high intensity mercury light was switched on to induce platelet aggregation photochemically. Appearance of first platelet aggregation and total blood flow stop were timed in seconds. Data was statistically analyzed using Mann-Whitney test. The results showed that in the animals treated with *Aloe vera* gel juice, venule as well as arteriole platelet aggregation timings were significantly delayed ( $p < 0.05$ ) in comparison to the controls [(control: venule 1st aggregation  $25.40 \pm 1.14$ , flow stop  $173.80 \pm 19.9$ ; arteriole: 1st aggregation  $56.00 \pm 8.51$ , flow stop  $140.40 \pm 8.93$ ; *Aloe vera* gel juice: venule 1st aggregation  $28.50 \pm 1.97$ , flow stop  $209.67 \pm 16.11$  arteriole: 1st aggregation  $103.50 \pm 7.31$ , flow stop  $166.17 \pm 19.63$ ]. Data shows the beneficial influence of *Aloe vera* gel juice delaying thrombus formation in the cerebral microvessels, *in vivo*. The delay in thrombus formation may be attributed to the presence of superior antioxidants present in the *Aloe vera* gel juice by protecting cells in the body against destruction by free radicals thus reducing the risk of arteriosclerosis<sup>[52]</sup>.

Five thousand patients of atheromatous heart disease, presented as angina pectoris were studied over a period of five years. After adding the 'Husk of Isabgol: *Plantago ovata* seed' and '*Aloe vera*' to the diet, a marked reduction in total serum cholesterol, serum triglycerides, fasting and post prandial blood sugar level in diabetic patients, total lipids and also increase in HDL were noted. Simultaneously the clinical profile of these patients showed reduction in the frequency of anginal attacks and gradually, the drugs, like verapamil, nifedipine,  $\beta$ -blockers and nitrates, were tapered. The patients, most benefited, were diabetics (without adding any antidiabetic drug). The exact mechanism of the action of the above two substances is not known, but it appears, that probably they act by their high fibre contents. Both these substances need further evaluation. The most interesting aspect of the study was that no untoward side effect was noted and all the five thousand patients are surviving till date<sup>[53]</sup>.

The authors electronically searched relevant studies in MEDLINE, CINAHL, Cochrane Library, Health STAR, DARE, South-

East Asia Database, Chinese Databases, and several Thai local Databases (1918-June, 2004). Only controlled clinical trials for burn healing were included. There were no restrictions on any language of publication. Two reviewers independently extracted data on study characteristics, patient characteristics, intervention, and outcome measure. Four studies with a total of 371 patients were included in this review. Based on a meta-analysis using duration of wound healing as an outcome measure, the summary weighted mean difference in healing time of the *Aloe vera* group was 8.79 days shorter than those in the control group ( $p = 0.006$ ). Due to the differences of products and outcome measures, there is paucity to draw a specific conclusion regarding the effect of *Aloe vera* for burn wound healing. However, cumulative evidence tends to support that *Aloe vera* might be an effective interventions used in burn wound healing for first to second degree burns. Further, well-designed trial with sufficient details of the content of *Aloe vera* products should be carried out to determine the effectiveness of *Aloe vera*<sup>[54]</sup>.

The authors examined herbal formulation having *Aloe vera* gel powder for its efficacy and activity on bed sores. *Aloe vera* gel powder with high molecular weight (AHM) was prepared from the gel part, by washing with running water using the patented freeze-drying under micro wave and far infra red irradiations in which barbaloin content was less than 10 ppm in the powder form. The treatment was given by applying AHM ointment for bed sores from 1 degree to 2 degree ulcer patients. The results have shown that AHM in the ointment form indicated a high possibility to cure bed sores. Being very difficult to cure, due to the patient's peculiar conditions such as old age, inability of the patient to turn by himself/herself and also due to complications caused by other symptoms. The authors were able to confirm the effectiveness of the aloe ointment in six cases of bed sores with two cases of positive control, using the Design Score and by checking the side effects. The authors reported the pre-clinical trials for bed sores by the external use of AHM ointment<sup>[55]</sup>.

The authors investigated the effects of acemannan (ACE), a polysaccharide extracted from *Aloe vera* gel, on gingival fibroblasts proliferation; keratinocyte growth factor (KGF)-1, vascular endothelial growth factor (VEGF) and type I collagen production; and oral wound healing in rats. <sup>3</sup>H-Thymidine incorporation assay and ELISA were used. Punch biopsy wounds were created at the hard palate of male Sprague Dawley rats. All treatments [normal saline; 0.1% triamcinolone acetonide; plain 1% Carbopol (a rheology modifier); Carbopol containing 0.5%, 1%, and 2% ACE (w/w)] were applied daily. Wound areas and histological features were observed at day 7 after treatment. From the studies, ACE at concentrations of 2, 4, 8, and 16 mg/ml significantly induced cell proliferation ( $p < 0.05$ ). Wound healing of animals receiving Carbopol containing 0.5% ACE (w/w) was significantly better than that of the other groups ( $p < 0.05$ ). These findings suggest that ACE plays a significant role in the oral wound healing process via the induction of fibroblast proliferation and stimulation of KGF-1, VEGF, and type I collagen expressions<sup>[56]</sup>.

The authors assessed the effects of *Aloe vera* cream in reducing postoperative pain, post-defection pain, and its promotion of wound healing after open hemorrhoidectomy. **Design:** A prospective, randomized, double-blind, placebo-controlled trail was conducted comparing the effects of a cream containing *Aloe vera* versus a placebo cream on post-hemorrhoidectomy pain. The study preparations were applied by patients to the surgical site 3 times per day for 4 weeks after hemorrhoidectomy. Pain was assessed with a visual analog scale immediately post-operatively and at hours 12, 24, and 48 after surgery and at weeks 2 and 4. Wound healing was

examined and evaluated at the end of 2 and 4 weeks. The use of analgesics was recorded. Results: Forty-nine patients were randomly assigned to receive aloe ( $n=24$ ) or placebo ( $n=25$ ). Patients in the topical aloe cream group had significantly less post-operative pain at hours 12, 24, and 48 hours and at 2 weeks. Aloe cream reduced the pain after defecation in 24 and 48 hours postsurgery ( $p<0.001$ ). **Conclusion:** Application of *Aloe vera* cream on the surgical site is effective in reducing post-operative pain both on resting and during defecation, healing time, and analgesic requirements in the patients compared with the placebo group<sup>[57]</sup>.

The randomized double-blind placebo-controlled clinical trial with hyper-lipidemic (hyper-cholesterolemic and/or hyper-triglyceridemic) type 2 diabetic patients aged 40 to 60 years not using other anti-hyperlipidemic agents and resistant to daily intake of two 5 mg glyburide tablets and two 500 mg metformin tablets, the efficacy and safety of taking aloe gel (one 300 mg capsule every 12 hours for 2 months) combined with the aforementioned drugs in treatment of 30 patients were evaluated and compared with the placebo group ( $n=30$ ). The aloe gel lowered the fasting blood glucose, HbA1c, total cholesterol, and LDL levels significantly ( $p=0.036$ ,  $p=0.036$ ,  $p=0.006$ , and  $p=0.004$ , respectively) without any significant effects on the other blood lipid levels and liver/kidney function tests ( $p>0.05$ ) compared with the placebo at the endpoint. No adverse effects were reported. The results suggest that aloe gel may be a safe anti-hyperglycemic and anti-hypercholesterolemic agent for hyperlipidemic type 2 diabetic patients<sup>[58]</sup>.

*Aloe vera* gel of 50% and 96.4% were tested for its wound healing activity by topical application in experimental rats. The effect of *Aloe vera* gel on wound healing was evaluated by wound excision model and histopathology was used to study the effect on wound healing. The effect produced by *Aloe vera* gel with reference to wound contraction, wound closure, decrease in surface area of wound, tissue regeneration at the wound site and histopathological characteristics were significant in treated rats. The effect of *Aloe vera* gel on biochemical studies revealed significant increase in collagen and decreased hexosamine content and malondialdehyde levels when compared with control. The present study thus provided scientific rationale for the traditional use of *Aloe vera* gel for management of wounds<sup>[59]</sup>.

The authors aimed to formulate and optimize a herbal gel of *Aloe vera* extract containing Carbopol 934 as gelling agent and to investigate the effects of topical application of Carbopol 934 gel containing *Aloe vera* extract on the healing of skin wound surgically induced in Wistar rats. **Materials and methods:** Different concentrations of viscosity enhancer Carbopol 934 were tried and finally gel that showed good spreadability and consistency was selected for wound healing property of herbal gel of *Aloe vera*. Excision wound model was used for the study. **Results:** The optimized gel was evaluated for different physico-chemical properties and wound healing property. Differences in wound healing were observed between the various treatments when compared to the control group. Tissue hyperplasia was lower in the control group compared to the other treated groups. In animals group treated with gel, 80.14% healing was observed up to 14<sup>th</sup> day. While in untreated group 1 (control) animals showed 52.68% healing of wound on 14<sup>th</sup> day. On the other hand, control group animal also showed inflammation and pus formation up to 5<sup>th</sup> day of study, while treated animal did not show any observable inflammation and pus formation. **Conclusion:** Results showed prepared gel has promising effect on the wound healing process<sup>[60]</sup>.

The authors aimed to assess the efficacy of *Aloe vera* gel compared

with 1% silver sulfadiazine cream (SSD) as a burn dressing for the treatment of superficial and partial thickness burns. **Methods:** This International Comparative study was carried out at the Burn unit and Plastic surgery department, Nishtar Hospital Multan, Pakistan from July 2008 to December 2010. A total of 50 patients with superficial and partial thickness burns were divided into two equal groups randomly by consecutive sampling method, one group was dressed with *Aloe vera* gel while the other was treated with SSD, and the results regarding duration of wound epithelialization, pain relief and cost of treatment were compared. **Results:** In patients treated with *Aloe vera* gel, healing of burn wounds were remarkably early than those patients treated with SSD. All the patients of *Aloe vera* group were relieved of pain earlier than those patients who were treated with SSD. **Conclusion:** Thermal burns patients dressed with *Aloe vera* gel showed advantage compared to those dressed with SSD regarding early wound epithelialization, earlier pain relief and cost-effectiveness<sup>[61]</sup>.

The present review is composed of positive/negative effect of *Aloe vera* for *in vitro/in vivo* r-ray radiation-induced tissue alterations, possible prophylactic efficacy of aloe polysaccharide for r-ray radiation-induced damages under subsections organized by the nature of protection modes, and *Aloe vera* phenolic inhibitors for insulin receptor. *In vitro/in vivo* insulin permeation enhancements by *Aloe vera* gel were clearly revealed from the viewpoint of drug delivery system, and the treatment of insulin resistant mice with the polyphenol-rich *Aloe vera* extract diminished their insulin resistance. Modified *Aloe vera* polysaccharides with aloe phenolic chromones, aloesin groups, decreased in insulin level observed in subjects in condition of pre-diabetic/metabolic syndrome. Therefore, the possible prophylactic efficacy of *Aloe vera* extract for r-ray radiation-induced skin damages may be resulted from synergistic effects taking place between phenolic ingredients; chromones and aloe polysaccharides as adjuvants<sup>[62]</sup>.

## BIOAVAILABILITY (DRUG DELIVERY SYSTEM) BY ALOE GEL

The authors reported the effect of *Aloe vera* preparations on human absorption of vitamin C and E, the most popular vitamin supplements. The plasma bioavailability of vitamins C and E were determined in normal fasting subjects, with eight subjects for vitamin C and ten subjects for vitamin E. In a random crossover design, the subjects consumed either 500 mg of ascorbic acid or 420 mg of vitamin E acetate alone (control) or combined with 2 oz of two different aloe preparations (a whole leaf extract or an inner fillet gel). Blood was collected periodically up to 24 h after consumption. Plasma was analyzed for ascorbate and tocopherol by HPLC with UV detection. There was no significant difference in the areas under the plasma ascorbate-time curves among the groups sincerely due to large differences within the groups. For comparative purposes the control area was 100%. The aloe gel area was 304%, and aloe whole leaf 80%. Only aloe gel caused a significant increase in plasma ascorbate after 8 and 24 h. For vitamin E, the results for the relative areas were control 100%, gel 369%, and leaf 198%. Only the aloe produced a significant increase in plasma tocopherol after 6 and 8 h. Both aloe were significantly different from the control after 8 h. Aloe gel was significantly different from the baseline after 24 h. The aloe slowed down the absorption of both vitamins with maximum concentrations 2-4 h later than the control. There was no difference between the two types of aloe. The results indicate that the aloe improve the absorption of both vitamins C and E. The absorption is

slower and the vitamins last longer in the plasma with the aloes. Aloe is the only known supplement to increase the absorption of both of these water- or fat-soluble vitamins and should be considered as a complement to them<sup>[63]</sup>.

The authors determined that the effect of *Aloe vera* gel (GEL) and whole leaf extract (WLE) on the permeability of Caco-2 cell monolayers. Solution of GEL and WLE were prepared to cell monolayers, and the transepithelial electrical resistance was monitored for 2 h, which was then continued for another 2 h after removal of the test solutions to measure reversibility of the effect. The transport of insulin in the presence and absence of GEL and WLE solutions was also investigated. Both extracts were able to significantly reduce the transepithelial electrical resistance of Caco-2 cell monolayers at concentrations above 0.5% w/v and thereby showed the ability to open tight junctions between adjacent cells. This effect was fully reversible, as the electrical resistance of the cell monolayers returned to the original value upon removal of the test solutions. The GEL and WLE solutions significantly enhanced the transport of insulin across the Caco-2 cell monolayers compared with the control. The results suggest that these plant products have a high potential to be used as absorption enhancers in novel dosage forms for drug with poor bioavailabilities when administered orally. On the other hand, an uncontrolled increase in the bioavailability of drugs that are taken simultaneously with GEL and WLE products may result in adverse effects, and the potential exists that toxic blood plasma levels may be reached<sup>[64]</sup>.

The aim of this study is to examine the effect of two different *Aloe vera* preparations (aloe inner leaf gel; AG and aloe whole leaf decolorized gel; AL) compared to placebo on the bioavailability of vitamins C and B<sub>12</sub>, in healthy human volunteers in a randomized crossover trial. Subjects ( $n=15$ ) received in a random fashion either aloe whole leaf extract (AL with vitamins B<sub>12</sub>, 1 mg and vitamin C, 500 mg) or aloe fillet gel (AG with B<sub>12</sub> 1 mg and vitamin C 500 mg) or water (with vitamin B<sub>12</sub> 1 mg and vitamin C 500 mg). Blood was obtained fasting, followed by 1, 2, 4, 6, 8, and 24 h post-ingestion of aloe/water. When given with vitamins C and B<sub>12</sub>, AG significantly increased plasma oxygen radical absorbance capacity at both 2 and 24 h and AL at 4 h compared to baseline and placebo. AG significantly increased plasma vitamin C at 4, 6, 8, and 24 h and AL at 4 and 6 h compared to baseline and placebo ( $p<0.01$ ). Also, both aloes significantly increased serum vitamin B<sub>12</sub> levels at 1 and 2 h compared to baseline and placebo ( $p<0.01$ ). Thus, AG and AL preparations are safe, well tolerated, and enhance the bioavailability of vitamins C and B<sub>12</sub> and antioxidant potential<sup>[65]</sup>.

The authors investigated the potential of other species of aloe to act as drug absorption enhancement agents. The effect of gel materials from three South African aloes; *Aloe ferox*, *A. marlothii* and *A. speciosa* on the transepithelial electrical resistance and permeability of atenolol, a selective  $\beta_1$ -receptor antagonist, across excised intestinal tissue of the rat as well as the transport of FITC-dextran across Caco-2 monolayers was investigated. The aloe gel materials exhibited the ability to statistically significantly reduce the transepithelial electrical resistance of excised rat intestinal tissue but did not significantly increase the transport of atenolol across this *in vitro* tissue model at the concentrations tested. At least one concentration of each aloe gel material enhanced the transport of FITC-dextran statistically significantly across Caco-2 cell monolayers. The aloe gel materials showed potential to act as drug absorption enhancing agents across intestinal epithelia. The absorption enhancement effect was dependent on the type of *in vitro* model and type of drug was investigated<sup>[66]</sup>.

The effect of gel and whole leaf materials from 3 different aloe species namely *A. ferox*, *A. marlothii*, and *A. vera* as well as polysaccharides precipitated from *Aloe vera* materials on the bi-directional transport cimetidine (a histamine H<sub>2</sub> receptor antagonist) across rat intestinal tissue was investigated. **Materials and methods:** Cimetidine transport studies were performed across excised rat intestinal tissue mounted in Sweetana-Grass diffusion chambers in both the apical-to-basolateral and basolateral-to-apical directions. **Results:** While *Aloe vera* gel and whole leaf materials did not inhibit the efflux of cimetidine, the polysaccharides precipitated from them did show a reduction of cimetidine efflux. On the other hand, both *A. ferox* and *A. marlothii* gel and whole leaf materials exhibited an inhibition effect on cimetidine efflux. **Conclusion:** This study identified a modulation effect of efflux transporters by certain aloe materials. This may cause herb-drug pharmacokinetic interaction when drugs that are substrates for these efflux transporters are taken simultaneously with aloe materials. On the other hand, these aloe materials may be used for drug absorption enhancement for drugs with low bioavailability due to extensive efflux<sup>[67]</sup>.

The authors developed an improved dressing film comprising 1.95% w/v fibroin and 0.05% w/v aloe gel extract. The tensile strength of dry film was 21±0.5 MPa and broke at 1.1±0.2% elongation; corresponding values for wet film were 18±1.3 MPa and 1.9±0.1% elongation. The film maintained its shape upon water immersion and the swelling ratio of the dry film was 0.8±0.1 while the water uptake was 43.7±2.6%. After 28 days of incubation in phosphate buffered saline (1 M, pH 7.4, 37°C), the weight of film was reduced by 6.7±1.1% and the tensile strength and elongation at breaking point (dry state) were 15.4±0.6 MPa and 1.5±0.2%, respectively. Compared to aloe-free fibroin film (2.0% fibroin extract only), the blended film enhanced the attachment and proliferation of skin fibroblasts. The bFGF immunofluorescence of fibroblasts cultured on the blended film appeared greater than those cultured on tissue culture plate or on aloe-free fibroin film while  $\alpha$ -smooth muscle actin was maintained. In streptozotocin-induced diabetic rats, the wound dressed with the blended film were smaller ( $p<0.05$ ) by day 7 after wounding, compared to untreated diabetic wound. Histology of repaired diabetic wound showed the fibroblast distribution and collagen fiber organization to be similar to wound in normal rats, and this was matched by enhanced hydroxyproline content. Thus, such accelerated wound healing by the blended fibroin/aloe gel films may find application in treatment of diabetic non-healing skin ulcers<sup>[68]</sup>.

The buccal mucosal route offers several advantages but the delivery of certain drugs can be limited by low membrane permeability. The authors investigated the buccal permeability properties of didanosine (ddI; anti-HIV drug) and assessed the potential of *Aloe vera* gel (AVgel) as a novel buccal permeation enhancer. Permeation studies were performed using Franz diffusion cells, and the drug was quantified by UV spectroscopy. Histomorphological evaluations were undertaken using light and transmission electron microscopy. The permeability of ddI was concentration-dependent, and it did not have any adverse effects on the buccal mucosae. A linear relationship ( $R^2=0.9557$ ) between the concentrations and flux indicated passive diffusion as the mechanism of drug transport. AVgel at concentrations of 0.25 to 2 %w/v enhanced ddI permeability with enhancement ratios from 5.09 (0.25%w/v) to 11.78 (2 %w/v) enhanced ddI permeability at 4 and 6 %w/v. Ultrastructural analysis of the buccal mucosae treated with phosphate buffer saline pH 7.4 (PBS), ddI/PBS, and ddI/PBS/AVgel 0.5%w/v showed cells with normal plasmalemma, well-developed cristae, and nuclei with regular nuclear envelopes. However, cells from 1, 2, and 6 %w/v AVgel-treated

mucosae showed irregular nuclear outlines, increased intercellular spacing, and plasmalemma crenulations. This study demonstrates the potential of AVG as a buccal permeation enhancer for ddI to improve anti-HIV and AIDS therapy<sup>[69]</sup>.

The aim of the study was to investigate the *in vitro* cytotoxicity of *Aloe vera*, *A. marlothii*, *A. speciosa* and *A. ferox* against human hepatocellular (HepG2), human neuroblastoma cells (SH-SY5Y) and human adenocarcinoma epithelial cells (HeLa). Flow cytometry was used to measure cell viability, apoptosis and reactive oxygen species (ROS). The aloe gel materials investigated only decreased cell viability at concentrations of >10 mg/mL and exhibited half-maximal cytotoxic concentration (CC50) values above 1.000 mg/mL, except for *Aloe vera* gel in HepG2 cells (CC50=269.3 mg/mL). *A. speciosa* whole-leaf material showed a significant decrease in viability of HeLa cells, whereas the other whole-leaf materials did not show a similar effect. The aloe gel materials caused a dose-dependent increase of apoptosis in HeLa cells. None of the aloe materials investigated exhibited a significant increase in ROS. It can be concluded that the selected aloe materials caused only limited reduction in cell viability with limited *in vitro* cytotoxicity effects. Further, neither significant apoptosis effects were observed nor induction of ROS<sup>[70]</sup>.

The aim of the work is to develop and evaluate polymeric film containing *Aloe vera* and vitamin E to treat wound caused by burn. Polymeric films containing different quantities of sodium alginate and polyvinyl alcohol were characterized for their mechanical properties and drug release. The polymeric films, which were produced, were thin, flexible, resistant, and suitable for application on damaged skin, such as in burn wound. Around 30% of vitamin E acetate was released from the polymeric films within 12 h. The *in vivo* experiments with tape stripping indicated an effective accumulation in the stratum corneum when compared to a commercial cream containing the same quantity of vitamin E acetate. Vitamin E acetate was found in higher quantities in the deep layers of the stratum corneum when the film formulation was applied. The results obtained show that the bioadhesive films containing vitamin E acetate and *Aloe vera* could be an innovative therapeutic system for the treatment of burn<sup>[71]</sup>.

## NEURO-STIMULATION AND INSULIN-SENSITIVITY OF ALOE VERA

The authors explored the efficacy of *Aloe vera* in animal modes of learning and memory, depression, and locomotion. **Methods:** To assess learning and memory, the passive avoidance task and elevated plus-maze were used. For evaluating depression, the forced swim test and tail suspension test were performed, and to assess locomotor activity, the rota rod test and photoactometer were used. **Results:** *Aloe vera* (200 mg and 400 mg/kg, p.o.) was found to significantly increase the acquisition and retention step-down latency as compared to control in the passive avoidance task. In the elevated plus-maze, the highest administrated dose (400 mg/kg, p.o.) of *A. vera* significantly reduced the transfer latency as compared to control. The forced swim test as well as tail suspension test showed that *A. vera* at all administrated doses (100, 200, and 400 mg/kg, p.o.) depressed the period of immobility significantly. However, the locomotor activity did not show any significant change in the rota rod test and photoactometer. **Discussion:** It can be proposed that *A. vera* enhances learning and memory, and also alleviates depression in mice<sup>[72]</sup>.

Recent prospective studies provided evidence that higher adherence to a Mediterranean-type diet could be associated with slower cognitive decline, reduced risk of progression from mild

cognitive impairment to Alzheimer's disease (AD), reduced risk of AD, and decreased mortality in AD patients. Cognitive disorders of aging represent a serious threat to the social and economic welfare of current society. It is now widely recognized that pathology related to such conditions, particularly AD, likely begins years or decades prior to the onset of clinical dementia symptoms. Insulin, a hormone with potent effects in the brain, has recently received a great deal of attention for its potential beneficial and protective role in cognitive function.

The present pilot study investigated the effect of an aloe polymannose multinutrient complex (APMC) formula on cognitive and immune functioning over 12 months (m) among adults diagnosed with Alzheimer's disease (AD). Subjects participated in an open-label trial and consumed 4-teaspoons/day of the APMC. The ADAS-cog, MMSE, ADCS-ADL, and SIB were administered at baseline and 3, 6, 9, and 12 m follow-up. Cytokines, lymphocyte, and monocyte subsets were assessed at baseline and 12 m. The mean ADAS-cog cognition score significantly improved at 9 and 12 m from baseline, and 46% of the sample showed clinically-significant improvement ( $\geq 4$ -point change) from baseline to 12 m. Participants showed significant decreases in tumor necrosis factor- $\alpha$ , vascular endothelial growth factor, and interleukins-2 and -4. CD90<sup>+</sup>, CD95<sup>+</sup>, CD3<sup>+</sup>, CD95<sup>+</sup>CD34<sup>+</sup>, CD95<sup>+</sup>CD90<sup>+</sup>, CD14<sup>+</sup>CD34<sup>+</sup>, CD14<sup>+</sup>CD90<sup>+</sup>, and CD14<sup>+</sup>CD95<sup>+</sup> decreased significantly, whereas CD14<sup>+</sup> significantly increased. Participants tolerated the APMC supplement with few, temporary adverse reactions. The results showed improvements in both clinical and physiological outcomes for disease that otherwise has no standard ameliorative remedy<sup>[73]</sup>.

The aim of the study was to evaluate whether certain plant polysaccharides can acutely improve mood and cognitive function. **Methods:** In a randomized, double-blind, placebo-controlled, between subjects design trial, 73 middle-aged adults consumed 4 g of a proprietary mixture of non-starch polysaccharides (NSP: *Aloe vera* powder, arabinogalactan, Ghatti gum, glucosamine HCl, Gum tragacanth, rice starch), a rice flour placebo, or a sucrose control. Participants completed testing at baseline and 30 min post-consumption. Acute effects of consumption on mood, cognition, and blood glucose were evaluated during mental tests designed to induce mental fatigue. **Results:** Significant improvement in recognition and working memory performance was observed in the group that consumed NSP compared with placebo or sucrose. Improvements in memory performance following NSP intake were independent of changes in blood glucose. **Discussion:** This is the first report of acute behavioural improvement following plant polysaccharides intake in healthy middle-aged adult under conditions of mental fatigue. The findings suggest that certain NSP may enhance memory performance through mechanism other than elevated blood glucose<sup>[74]</sup>.

By using the patented hyper-dry system after washing out coloured materials with running water, aloe high molecular fractions (AHM) were obtained from *Aloe vera* gel in original and natural form containing less than 10 ppm of barbaloin. AHM mainly contained high molecular fractions; polysaccharide (acemannan) and glycoprotein (verectin), showing immunomodulatory and anti-inflammatory activities<sup>[75]</sup>. On the basis of chemical and biochemical properties, AHM were examined for therapy designed by implementation of well-controlled preliminary clinical trials, and exhibited the efficacy as immunomodulators for viral infection-induced hepatic periportal fibrosis and type 2 diabetic patients, and as wound and ulcer-healing ointment to patients suffering from bed sores. Therefore, AHM were considered to be a novel low-cost and safe drug of natural origin, indicating a possible therapeutic efficacy



in prevention of age-related diseases<sup>[11]</sup>.

Insulin resistance, which refers to the reduced sensitivity of target tissues to the favorable effects of insulin, is related to multiple chronic conditions known to impact cognition and increase dementia risk. As a result, novel therapeutic strategies that focus on increasing insulin sensitivity in the brain may be an important target for protecting or treating cognitive decline.

The author highlighted the current understanding of the relation between insulin and aloe vera in human, potential mechanisms underlying the link between insulin resistance and dementia, and current experimental therapeutic strategies aimed at improving cognitive function via modifying the brain's insulin sensitivity. Diet metabolism and immunomodulatory activity are linked to both to each other, allowing mammals to adapt to diverse changes in their intestinal gut surroundings. The obesity as a primary source of disease brings about metabolic dysfunction followed by inflammatory insulin resistance. The metabolites of aloe polymannose moiety; a mannooligosaccharide and short chain fatty acids, synergistically modulated insulin sensitivity on tissues with combination of phenolics, such as aloesin, aloe emodin (a metabolite of barbaloin) and salicylate in *Aloe vera* gel. The insulin levels and insulin activity in the central nerve system such as hippocampus, are reduced in Alzheimer's disease (AD) and amnesic mild cognitive impairment (MCI). Restoring insulin levels to normal in the brain may provide therapeutic benefit to elderly subjects with AD and amnesic MCI. The effect of aloe polymannose multinutrients complex (APMC) on cognitive and immune functioning in AD showed the improvements in both clinical and physiological outcomes, though the several limitations of the current investigation were noted. APMC may offer an alternative opinion for persons with AD and amnesic MCI. Present review showed a possible putative efficacy of *Aloe vera* gel metabolites in long-term ingestion to insulin sensitivity<sup>[10]</sup>.

The authors presented highlights key mediators and mechanisms responsible for the link between endothelial dysfunction, insulin resistance and aging. In particular, the authors discussed the sirtuin-1 system, the p66Shc pathway, telomeres, and their interrelationships with endothelial damage and repair in the present review<sup>[76]</sup>.

## BORN FORMATION (BORN MARROW STROMAL CELLS) AND HUMORAL IMMUNE RESPONSE AUGMENTATION OF ALOE VERA GEL (ACEMANNAN)

The authors examined pulp structure, isolated structural components and determined their carbohydrate compositions along with analyzing a partially purified pulp-based product (acemannan hydrogel) used to make Carrisyn hydrogel wound dressing. Light and electron microscopy showed that the pulp consisted of large clear mesophyll cells with a diameter as large as 1,000 microm. These cells were composed of cell walls and cell membranes along with a very limited number of degenerated cellular organelles. No intact cellular organelles were found in mesophyll cells. Following distribution of pulp by homogenization, three components were isolated by sequential centrifugation. They were thin clear sheets, microparticles and a viscous liquid gel, which corresponded to cell wall, degenerated cellular organelles and liquid content of mesophyll cells based on morphological and chemical analysis. These three components accounted for 16.2% ( $\pm 3.8$ ), 0.70% ( $\pm 0$ ) and 83.1% of the pulp on a dry weight basis. The carbohydrate composition of each component was distinct; liquid gel contained mannan, microparticles

contained galactose-rich polysaccharide(s) and cell walls contained an unusually high level of galactouronic acid (34% w/w). The same three components were also found in acemannan hydrogel with mannan as the predominant component. Thus, different pulp structural components are associated with different polysaccharides and thus may potentially be different functionally. These findings may help lay a basis for further studies and development of better controlled progressing methods and applications for this well-accepted medicinal plant<sup>[77]</sup>.

The authors investigated the effect of acemannan isolated from *Aloe vera* gel on the steady level of dentin sialoprotein (DSPP) and dentin matrix protein 1 (DMP1) mRNA in primary human pulpal cells. **Materials and methods:** Cells were treated with the designated concentrations of acemannan (0.25, 0.5 and 1 mg/mL) for 24 h. The reverse transcription-polymerase chain reaction assays were used to investigate the effects of acemannan on the steady level of DSPP and DMP1 mRNA. **Results:** At 24 h of incubation, acemannan (0.5 mg/mL) significantly enhanced the expressions of DSPP and DMP1 mRNA levels up to 1.93 and 2.76 fold, respectively, as compared with the control group ( $p < 0.05$ ). **Conclusion:** Acemannan at concentration 0.5 mg/mL stimulated both DSPP and DMP1 mRNA expressions in human dental pulp cells<sup>[78]</sup>.

The tendon is composed of highly organized collagen fibers that form a complex supra-molecular structure. After lesions, the organization and composition of the tendon are not completely restored. The authors aim to evaluate if application of *Aloe vera* improves tendon healing, considering the effectiveness in the stimulus of collagen synthesis. **Methods:** The calcaneal tendon of male Wistar rats was partially transected with subsequent topical application of *Aloe vera* ointment at the injury. The animal were separated into groups with tendons treated with *Aloe vera* extract for 7 days and excised on the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days after surgery, control rats received only ointment base without plant extract. **Findings:** Morphological analysis using polarization microscopy showed that the entire tendon undergoes a remodeling process, with disorganized collagen fibers by days 7 and 14 in plant-treated and non-treated groups and with a higher birefringence in tendons of the plant-treated group on the 21<sup>th</sup> day. A higher concentration of hydroxyproline was found in plant-treated tendons on days 7 and 14 compared with their control. Western blots showed lower amounts of type I collagen in the plant-treated group on day 14 compared with the control. MMP-9 diminished 14 days after lesion and the active isoform of MMP-2 increased on day 21 in plant-treated groups. **Significance:** The present study indicates a beneficial effect of *Aloe vera* in the tissue reorganization in the transected region of the tendon 21 days after injury and is supported by an increase of active MMP-2<sup>[79]</sup>.

The authors hypothesized acemannan could affect bone formation and examined. Primary rat bone marrow stromal cells (BMSCs) were treated with various concentrations of acemannan. New DNA synthesis, VEGF, BMP-2, alkaline phosphatase activity, bone sialoprotein, osteopontin expression, and mineralization were determined by <sup>3</sup>H-thymidine incorporation assay, ELISA, biochemical assay, Western blotting, and alizarin red staining, respectively. In an animal study, mandibular right incisors of male Sprague-Dawley rats were extracted and an acemannan treated sponge was placed in the socket. After 1, 2, and 4 weeks, the mandibles were dissected. Bone formation was evaluated by dual-energy X-ray absorptiometry and histopathological examination. The *in vitro* results revealed acemannan significantly increased BMSCs proliferation, VEGF, BMP-2, alkaline phosphatase activity, bone sialoprotein and osteopontin expression, and mineralization. *In vivo*

results showed acemannan-treated groups had higher bone mineral density and faster bone healing compared with untreated controls. A substantial ingrowth of bone trabeculae was observed in acemannan-treated groups. These data suggest acemannan could function as a bioactive molecule inducing bone formation by stimulating BMSCs proliferation, differentiation into osteoblasts, and extracellular matrix synthesis. Acemannan could be a candidate natural biomaterial for bone regeneration<sup>[80]</sup>.

The authors explored the effect of aqueous extract of *Aloe vera* on parameters of humoral and cell-mediated immunity. **Materials and methods:** Delayed-type hypersensitivity in rats was assessed by measuring foot pad thickness following sensitisation by keyhole limpet haemocyanin injection and subsequently challenged by the same. Humoral immunity was assessed by measurement of haemagglutination titer to sheep red blood cells. **Results:** *Aloe vera* extract (400 mg/kg, p.o.) produced a significant decrease in foot pad thickness compared with the control group, and also significantly enhanced the secondary humoral immune response. **Conclusion:** These findings suggest that *Aloe vera* can modulate immune response by augmenting secondary humoral immunity and decreasing cell-mediated immunity<sup>[81]</sup>. The extract of *Aloe vera* influences both cell-mediated and humoral immunity in animals and these effects could be responsible for its role as an agent to combat various fungal infections and inflammatory conditions like atopic dermatitis and irritable bowel disease.

The authors evaluated the anti-inflammatory effects of aloe components (aloin, aloesin and aloe gel) known to be biologically active in the rat model of colitis. **Main methods:** Male Sprague Dawley rats were fed experimental diets for 2 weeks before and during the induction of colitis. Drinking water containing 3% dextran sulfate sodium (DSS) was provided for 1 week to induce colitis. At the end of the experimental period, clinical and biochemical markers were compared. **Key Findings:** Plasma leukotriene B<sub>4</sub> and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) concentrations were significantly decreased in all groups supplemented with aloe components compared to the colitis control group ( $p < 0.05$ ). Animals fed both a 0.1% and 0.5% aloesin supplemented diet showed colonic myeloperoxidase activities which were decreased by 32.2% and 40.1%, respectively ( $p < 0.05$ ). Colonic mucosa TNF- $\alpha$  and interleukin-1 $\beta$  mRNA expressions were significantly reduced in all animals fed aloin, aloesin, or aloe-gel ( $p < 0.05$ ). **Significance:** Dietary supplementation of aloe components ameliorates intestinal inflammatory responses in a DSS-induced ulcerative colitis rat model. In particular, aloesin was the most potent inhibitor<sup>[82]</sup>.

The authors reported the immunostimulatory and protective effects of *Aloe vera* extracts (aqueous and ethanolic) against coccidiosis in industrial broilers chickens. The study was divided into two experiments. Experiment-1 was conducted for the evaluation of immunostimulatory activity of *Aloe vera* extracts and experiment-2 demonstrated the protective efficacy of *Aloe vera* extracts against coccidiosis in chickens. Results of the experiment-1 revealed significantly higher ( $p < 0.05$ ) lymphoproliferative responses in chickens administered with ethanolic extract of *Aloe vera* as compared to those administered with aqueous extract and control group. Microplate haemagglutination assay for humoral response on day 7<sup>th</sup> and 14<sup>th</sup> post primary and secondary injections of sheep red blood cells (SRBCs) revealed significantly higher ( $p < 0.05$ ) anti SRBCs antibody (total IGs; IgG and IgM) titers in chickens of experimental groups as compared to the control group. None of the extracts, however, demonstrated significant effects on the development of lymphoid organs. Results of experiment-2 revealed

maximum protection (60%) in chickens administered with aqueous *Aloe vera* extract as compared to the ethanolic extract administered chicken (45%). Mean oocysts per gram of droppings in the control group was significantly higher ( $p < 0.05$ ) as compared to the chickens in both the experimental groups. Chickens administered with aqueous *Aloe vera* extract showed a minimal mean lesion score (2.3) followed by those administered with ethanolic *Aloe vera* extract (2.6) and control chickens (3.05) for caeca, and a similar pattern was observed for intestinal lesion scoring. Further, significantly higher weight gains and antibody titers ( $p < 0.05$ ) were observed in chickens administered with *Aloe vera* extracts as compared to those in the control group. It was concluded that *Aloe vera* may be a potential and variable candidate to stimulate the immune responses and can be used successfully as an immunotherapeutic agent against coccidiosis in industrial broiler chickens<sup>[83]</sup>.

## EFFECT OF ALOE VERA SUPPLEMENTATION IN SUBJECTS WITH PRE-DIABETIC/METABOLIC SYNDROME

The authors examined the effect of aloe compared to placebo on fasting blood, glucose, lipid profile, and oxidative stress in subjects with prediabetes/metabolic syndrome. **Methods:** This was a double-blind, placebo-controlled Institutional Review Board- approved pilot study of two aloe products (UP780 and AC952) in patients with prediabetes over an 8-week period. A total 45 subjects with impaired fasting glucose or impaired glucose tolerance and having two other features of metabolic syndrome were recruited ( $n = 15$ /group). Parameters of glycemia [fasting glucose, insulin, homeostasis model assessment (HOMA), glycosylated hemoglobin (HbA<sub>1c</sub>), fructosamine, and oral glucose tolerance test] and oxidative stress (urinary F<sub>2</sub>-isoprostanes) were measured along with lipid profile and high-sensitivity C-reactive protein levels before and after supplementation. **Results:** There were no significant baseline differences between groups. Compared to placebo, only the AC952 *Aloe vera* inner leaf gel powder resulted in significant reduction in total and low-density lipoprotein cholesterol levels, glucose, and fructosamine. In the UP780 *Aloe vera* inner leaf gel powder standardized with 2% aloesin group, there were significant reductions in HbA<sub>1c</sub>, fructosamine, fasting glucose, insulin, and HOMA. Only the UP780 aloe group had a significant reduction in the F<sub>2</sub>-isoprostanes compared to placebo. **Conclusion:** Standardized aloe preparations offer an attractive adjunctive strategy to revert the improved fasting glucose and impaired glucose tolerance observed in conditions of prediabetes/metabolic syndrome<sup>[84]</sup>.

The authors investigated to determine the effects of aloe QDM complex on body weight, body fat mass (BFM), fasting blood glucose (FBG), fasting serum insulin, and homeostasis model of assessment-insulin resistance (HOMA-IR) in obese individuals with prediabetes or early DM who were not on diabetes medications. **Methods:** Participants ( $n = 136$ ) were randomly assigned to an intervention [Aloe QDM complex: a 700 mg soft capsule compound of processed *Aloe vera* gel 147 mg/cap and aloesin powder (95% aloesin) 3 mg/cap, yeast chromone 125 mg/cap, and excipients; soybean oil, yellow beeswax, and lecithin, 425 mg/cap] or a control group [a 700 mg soft capsule of natural pigment 4.2 mg/cap and excipients (soybean oil, yellow beeswax, and lecithine 695.8 mg/cap)] and evaluated at baseline and at 4 and 8 wk. **Results:** The study lost six patients in the control group and eight in the intervention group. At 8 wk, body weight ( $p = 0.02$ ) and BFM ( $p = 0.03$ ) were significantly lower

in the intervention group. At 4 wk, serum insulin level ( $p=0.04$ ) and HOMA-IR ( $p=0.047$ ) were lower in the intervention group; they also were lower at 8 wk but with borderline significance ( $p=0.09$ ;  $p=0.08$ , respectively). At 8 wk, FBG tended to decrease in the intervention group ( $p=0.02$ ), but the between-group difference was not significant ( $p=0.16$ ). **Conclusion:** In obese individuals with prediabetes or early untreated DM, aloe QDM complex reduced body weight, BFM, and insulin resistance<sup>[85]</sup>.

*Aloe vera* is one of the oldest known medicinal plant but it is now realized that many of its active constituents may be addressed in different ways by different formulations. Several reputable suppliers produce a stabilized aloe gel for use as itself for formulations and there may be moves towards isolating and eventually providing verified active ingredients in dosable quantities. This review is aimed to discuss different extraction techniques for extracting bioactive compounds from *Aloe vera*<sup>[86]</sup>.

The authors aimed to assess 1-year efficacy and safety of salsalate (salicylate), containing in *Aloe vera* gel, in type 2 diabetes mellitus (T2DM). **Design:** Placebo-controlled, parallel trial; computerized randomization and centralized allocation, with patients, providers, and researchers blinded to assignment. (ClinicalTrials.gov: NCT00799643). **Setting:** 3 private practices and 18 academic centers in the USA. **Patients:** Persons aged 18 to 75 years with fasting glucose levels of 12.5 mmol/L or less ( $\leq 225$  mg/dL) and hemoglobin A1c (HbA1c) levels of 7.0% to 9.5% who were treated for diabetes. **Intervention:** 286 participants were randomly assigned (between Jan 2009 and July 2011) to 48 wk of placebo ( $n=140$ ) or salsalate, 3.5 g/d ( $n=146$ ), in addition to current therapies, and 283 patients were analyzed (placebo,  $n=137$ ; salsalate,  $n=146$ ). **Measurements:** Change in hemoglobin A1c level (primary outcome) and safety and efficacy measures. **Results:** The mean HbA1c level over 48 wk was 0.37% lower in the salsalate group than in the placebo group (95%CI, -0.53% to -0.21%;  $p<0.001$ ). Glycemia improved despite more reductions in concomitant diabetes medications in salsalate recipients than in placebo recipients. Lower circulating leukocyte, neutrophil, and lymphocyte counts show the anti-inflammatory effects of salsalate. Adiponectin and hematocrit levels increased more and fasting glucose, uric acid, and triglyceride levels decreased with salsalate, but weight and low density lipoprotein cholesterol levels also increased. Urinary albumin levels increased but reversed on discontinuation; estimated glomerular filtration rates were unchanged. **Limitation:** Trial duration and number of patients studied were insufficient to determine long-term risk-benefit of salsalate in T2DM. **Conclusion:** Salsalate (salicylate) improves glycemia in patients with T2DM and decreases inflammatory mediators. Continued evaluation of mixed cardiorenal signals is warranted<sup>[87]</sup>.

## THERAPEUTIC IMPLICATIONS

### Placenta extract

The hydrolysate of human placental extract (HPE) has been clinically approved for improvement of liver function in injection in Japanese and improvement of menopausal systems and fatigue of HPE injection was determined on randomized, single-blind, and case-controlled study design on healthy status in elderly Koreans<sup>[47]</sup>.

The extracts of placental lysates reduced the malignancy of a variety of human tumor cell lines in a species-unrestricted manner. Using a standard model of leukemia cell differentiation, the authors demonstrated that addition of placental extracts to tumor cells, or co-culture of tumor cells with the CD34<sup>+</sup> cells from umbilical cord blood, induced tumor cell differentiation. Inhibition of tumor growth

and metastasis *in vivo* was also observed following administration of placental extracts. These data support the concept of non-toxic biological therapy of cancer using stem cell derivatives, possibly through the induction of tumor cell differentiation<sup>[88]</sup>.

The influence of adult stem cells on tumor growth is paradoxical. On one hand, angiogenic factors secreted by stem cells are known to be essential for tumor vascularization. On the other hand, stem cell-derived factors can reportedly induce tumor differentiation or direct death of tumor cells. Both the placenta and umbilical cord are rich sources of stem cells with immune modulatory and tissue-healing properties; however, the effects of placental components on cancer cells have not been fully defined.

The authors reviewed the relevant literature concerning the main stem cells that populate the placenta. **Areas of agreement:** Recently, the placenta has become a useful source of stem cells that offer advantages in terms of proliferation and plasticity when compared with adult cells and permit to overcome the ethical and safety concern inherent in embryonic stem cells. In addition, the placenta has the advantage of containing epithelia, haematopoietic and mesenchymal stem cells. These stem cells possess immunosuppressive properties and have the capacity to suppress *in vivo* inflammatory responses. **Areas of controversy:** Some studies describe a subpopulation of placenta stem cells expressing pluripotency markers, but for other studies, it is not clear whether pluripotent stem cells are present during gestation beyond the first few weeks. Particularly, the expression of some pluripotency markers such as SSEA-3, TRA-1-60 and TRA-1-81 has been reported by the authors, but not by others. **Growing points:** Placenta stem cells could be of great importance after delivery for banking for autologous and allogeneic applications. The beneficial effects of these cells may be due to secretion of bioactive molecules that act through paracrine actions promoting beneficial effects. **Areas timely for development research:** Understanding the role of placenta stem cells during pregnancy and their paracrine actions could help in the study of some diseases that affect the placenta during pregnancy<sup>[89]</sup>.

Defibrotide for prophylaxis of hepatic veno-occlusive disease in paediatric haematopoietic stem-cell transplantation (Hsct) showed a useful clinical option for this serious complication of Hsct on an open-labelled, phase 3, and randomised controlled trial<sup>[16]</sup>.

Gel-repairer is a biomaterial composed of defibrotide, heat shock proteins and a thickening substance. It works as a local mesenchymal stem cells (Mscs) stimulator, finally generating connective tissue renewal. The research is within the field of regenerative medicine and has historically built its foundation from the studies carried out on non-vital amnion and placental membranes. The end point is the activation and stimulation of the local Mscs for the structural recovery of the joint involved in the degenerative process. Since 2003, the authors have been applying the gel repairer over more than 1,200 patients, most of them elderly, affected by degenerative joint disease. After 10 years of clinical experience, the results are really impressive, including the absence of toxicity, adverse reactions or side effects. The clinical findings allowed the presentation of a clinical preliminary study performed on a large group of patients from 2003 to 2009. The mechanism of action of the Joint Self-Repair procedure and some new technical opportunities were presented on tissue engineering advances in this fast evolving sector<sup>[90]</sup>.

Central and peripheral administration of kisspeptin (KP) to mammals stimulates gonadotrophin secretion via gonadotrophin releasing hormone stimulation. Similar observations have been reported in human studies as well as an increase in luteinizing hormone pulsatility. KP is now known to be associated with brain

sexual differentiation, sexual dimorphism, pubertal initiation and sex steroids feedback loops. Metabolic state, stress, and other neuropeptides such as neurokinin B are associated with changes in kisspeptin's stimulatory action. The conclusions from KP studies so far have led to the consideration of potential therapeutic applications. Increasing the understanding of KP may aid the authors's knowledge and management of infertility, contraception and hormone sensitive conditions<sup>[91]</sup>.

KPs are a group of newly discovered peptides, which have been found to play an important regulatory role in human reproduction. Loss of function mutations of KP or the KP receptor have been shown to cause pubertal failure; whereas activating mutations cause precocious puberty. In a recent study KP peptides were suggested to have not only a reproductive hormone properties, but neuro-protective properties against A $\beta$  plus related amyloid proteins. The release of KP from human neuronal cells has been shown to be stimulated by A $\beta$  suggesting that in regions that express the KiSS-1 gene, which encodes for the KP peptides, there may be changes in KP levels in AD due to the elevations of A $\beta$ <sup>[27]</sup>.

Further drug-drug interaction study targeting between JBP485, dipeptide, and cancer preventive drug is expected in the field of gastroenterology and hepatology.

#### *Aloe vera*

Aloe plant is rich in vitamins, minerals, enzymes, sugars etc, however, most of its health benefits have been attributed to the polysaccharides found in the gel of leave. *Aloe vera* plant has strong antibacterial, antifungal, antiviral, antioxidant, antiulcer and antitussive activity which emerged through the research done by various workers. In present review the authors compiled some properties of *Aloe vera* with its medicinal uses<sup>[92]</sup>.

Verectin, a glycoprotein fraction was obtained in a ratio of 20% in aloe high molecular fraction (AHM) by ELISA. Verectin, a radical scavenging glycoprotein in AHM, inhibited cyclooxygenase-2 and thromboxane A2 synthase, suggesting the participation to human immune system. The verectin-derived N-terminal octapeptide (Asp-Glu-Asp-Asn-Val-Leu-Leu) exerted a significant *in vivo* inhibiting effect on the growth of tumor (Ehrlich ascites carcinoma cells progression), which correlated with the prolongation of animal's life span. The study of AHM with verectin and acemannan provides evidence that AHM could be used as biodegradable safe and non-toxic adjuvants<sup>[75]</sup>.

The authors investigated the effect of *Aloe vera* whole leaf extract on pure and mixed human gut bacterial cultures by assessing the bacterial growth and changes in the production of short chain fatty acids. **Methods and Results:** *Bacteroides fragilis*, *Bifidobacterium infantis*, and *Eubacterium limosum* were incubated with *Aloe vera* extracts [0%, 0.5%, 1%, 1.5%, and 2%; (w/w)] for 24 and 48 h. Short chain fatty acids production was measured by gas chromatography/mass spectrometry analyses. A significant linear increase in growth response to *Aloe vera* supplementation was observed at 24 h for each of the bacterial cultures; however, only *B. infantis* and a mixed bacterial culture showed a significant positive linear dose response in growth at 48 h. In pure bacteria cultures, a significantly enhanced dose response to *Aloe vera* supplementation was observed in the production of acetic acid by *B. infantis* at 24 h and of butyric acid by *E. limosum*, at 24 and 48 h. In the mixed bacterial culture, the production of propionic acid was reduced significantly at 24 and 48 h in a dose- dependent fashion, whereas butyric acid production showed a significant linear increase. **Conclusion:** The results indicated that *Aloe vera* possessed bacteriogenic activity *in vitro* and

altered the production of acetic, butyric and propionic acids by micro-organisms selected for the study. Significance and impact of the study: The results of the study suggest that consumption of a dietary supplement, *Aloe vera*, may alter the production of short chain fatty acids by human intestinal microflora<sup>[93]</sup>. The metabolites of aloe polymannose moiety, such as a manno oligosaccharide and short chain fatty acids, synergistically may modulate insulin sensitivity on tissues with combination of phenolics, such as aloesin, barbaloin and salicylate in *Aloe vera* gel<sup>[10]</sup>.

Much of attention on diet patterns and Alzheimer's disease or cognition among the elderly has focused on the role of single nutrients or foods. The inherent advantages as well as the existing evidence of dietary pattern analyses strongly suggest that this approach may be valuable in AD and aging research.

The recent findings in the rapidly expanding field such as cognitive decline in elderly people were reviewed. Some nutrients, such as vitamins and fatty acids, have been studied longer than others, but strong scientific evidence of an association is lacking even for these compounds. Specific dietary patterns, like the Mediterranean diet, may be more beneficial than a high consumption of single nutrients or specific food items. A strong link between vascular risk factors and dementia has been shown, and the association of diet with several vascular and metabolic diseases is well known. Other plausible mechanisms underlying the relationship between diet and cognitive decline, such as inflammation and oxidative stress, have been established. In addition to the traditional etiological pathways, new hypotheses, such as the role of the intestinal microbiome in cognitive function, have been suggested and warrant further investigation<sup>[94]</sup>.

The nervous system and the immune system are two main regulators of homeostasis in the body. Systematically, proper functioning of the immune system is critical for maintaining normal nervous system function. Disruption of the immune system functioning leads to impairments in cognition and in neurogenesis. The communication between the nervous and the immune systems in the interest of normal central nervous system development and function is essentially required to ensure normal functioning of the organism. An aloe multinutrient complex formula may not only facilitate cognitive improvement, but also improve the inflammatory and immune functioning profile as well, thereby enhancing host recovery and improving overall quality of life.

## CONCLUSION AND FUTURE PERSPECTIVES

Animal and human tissues, such as placenta, and plant materials, such as aloe, under the stressed conditions had widely been investigated as original and clinical sources of the so-called "biogenic stimulant", producing a biological rejuvenescence.

Human placental extract has been used in oriental medicine as a restorative agent. The recent findings of the physiological function and pre-clinical application with polydeoxy ribonucleotide (Defibrotide) and metastin, a novel effective therapy for cancer metastasis, from placental extract, and an immunomodulatory therapy utilizing human placenta-derived progenitor/stem cells as a source for clinical study possibly showed the potential to provide disease-modifying or even curative outcomes for serious diseases.

Defibrotide prophylaxis seems to reduce incidence of veno-occlusive disease and could present a useful clinical option for the serious complication of haemopoietic stem-cell transplantation. A clinical trial using PDA001, an immunomodulatory therapy utilizing human placenta-derived stem cells, begins the initiation of phase I in U.S.A. for patients with moderate-to-severe Crohn's disease, a



chronic inflammatory condition of the gastrointestinal track, who are refractory to oral corticosteroids and immune suppressants. Further exploration of placenta in intractable disease is fully expected in near future. JBP485, a novel dipeptide isolated from human placental hydrolysate, was recognized by transporter, resulting in its rapid and complete absorption on drug transport system(s). JBP485 also repaired liver function after iv and oral administrations, suggesting an anti-hepatitis effects on cultured hepatocytes. Further clinical applications of JBP485 as a potent anti-hepatitis reagent are fully expected.

Gut microbiota now appears to influence the host at nearly every level and in every organ system, highlighting the interdependence and coevolution. Its adaptation to our changing life-style, such as diet-associated differences in gut microbiota composition, is astonishing, and highlighting that the consequences of our behaviours affect not only the environment without, but also that within us. Diet and placental extract- and *Aloe vera*-supplementation in particular have become the object of intense research in relation to cognitive aging and neuro-degenerative disease. Microbial colonization of mammals is an evolution-driven process that modulates host physiology, many of which are associated with immunity and nutrient intake. The microbial colonization process initiates signaling mechanisms that affect neuronal circuits involved in motor control and anxiety behavior. It is hypothesized that the normal gut microbiota seeing in specific pathogen free mice, is an integral part of the external environmental signals that modulates brain development and function. Diet functional foods and gut microbiota transplantation are areas that have yielded some therapeutic success in modulating the gut microbiota. Further understanding of the importance of developing and maintaining gut microbiota diversity, and investigation of their effects on placental extract and *Aloe vera* supplementation may lead to targeted interventions for health promotion, disease prevention and management, and improvement of quality of life for patients on various age-related disease states.

The highlight of putative prophylaxes updated of human placental extract and *Aloe vera* was summarized on the basis of the concept as biogenic stimulants and under the stand points of gastroenterology and hepatology research in the present review.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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