

## Putative Prophylaxes of Aloe Vera for Age-Related Diseases

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### ABSTRACT

The aim of the present review is focused on putative prophylaxes of *Aloe vera* for age-related diseases, and its check items are generally known traditional and specific diseases followed by *In vitro*, animal, and preliminary clinical studies: (1) prevention of cancer and alleviation of progeria; (2) prevention of cardiovascular and cerebro-vascular disorders, and inhibition of acetylcholinesterase; (3) preliminary clinical study of an aloemannose multinutrient complex on cognitive and immune functioning in Alzheimer's disease; (4) prevention of photo-aging and polycystic ovarian syndrome; (5) stimulation of periodontal fibroblasts, bone formation, and healing of pain and nephro-toxicity; and (6) anti-inflammation and immuno-response, excluding diabetic and hepatic disorders.

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**Key words:** Putative prophylactic efficacies; *Aloe vera*; Age-related diseases

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### INTRODUCTION

Longevity depends on an important fitness trait that is governed by genetically inherited and environmentally acquired situations with participation of internal gut microbiota. *Aloe vera* or resveratrol supplementation in larval diet delayed adult aging in the fruit fly, *drosophila melanogaster*. The longevity extension seems to be effected by multi-functional mechanisms involving efficient lipid utilization, prevention of neurodegeneration, regeneration of nerve fibers, and upregulation of antioxidant enzymes<sup>[1]</sup>. Long term *Aloe vera* ingestion increased longevity, decreased the occurrence and severity of age-related diseases, and attenuated the age-associated physiological decline<sup>[2]</sup>. In earlier reports focusing on age-related diseases of *Aloe vera* gel fractions, preliminary therapeutic efficacies of *Aloe vera* gel high molecular weight fractions for preliminary clinical treatments of type 2 diabetes<sup>[3]</sup>, bed sores<sup>[4]</sup>, hepatic fibrosis<sup>[5]</sup>, and oral lichen planus<sup>[6]</sup> were widely evaluated. *Aloe vera* gel high molecular fraction exhibited immunomodulating responses as carbohydrate-based adjuvants, and possible efficacies of *Aloe vera* gel metabolites in long-term ingestion to insulin sensitivity were discussed<sup>[7]</sup> including the metabolism of barbaloin (aloin) and aloesin in *Aloe vera* by intestinal bacteria<sup>[8]</sup>.

Putative prophylactic efficacies of *Aloe vera* for age-related diseases were presented by generally known traditional and specific diseases following main checkup items: (1) prevention of cancer and alleviation of progeria; (2) prevention of cardiovascular and cerebro-vascular disorders, and inhibition of acetylcholinesterase; (3) preliminary clinical study of an aloemannose multinutrient complex on cognitive and immune functioning in Alzheimer's disease; (4) prevention of photo-aging and polycystic ovarian syndrome; (5) stimulation of periodontal fibroblasts, bone formation and healing of pain and nephrotoxicity; and (6) anti-inflammation and immuno-response. The present review excluded diabetic and hepatic disorders, because the preliminary clinical possible efficacies of *Aloe vera* high molecular fractions containing acemannan and glycoprotein, verectin, were reported in an earlier paper on diabetic<sup>[3]</sup> and hepatic disorders<sup>[5]</sup>.

Main putative prophylaxis of aloe was separated by subsection on *In vitro*, animal, human consumption, and preliminary clinical tests of the aloe composition in various preparations.

## PREVENTION OF CANCER AND ALLEVIATION OF PROGERIA

### *In vitro* and animal studies

It is well-accepted paradigm that environmental and lifestyle-related factors play a critical role in development of 90% of all cancers. There is a growing evidence which is highly suggestive that chronic inflammation is a critical mediator of various aspects in development of cancer linked in multiple faces. The mechanisms by which the risk factors induce cancer are becoming increasingly evident and one major process that seems to be common between the risk factors is inflammation<sup>[9]</sup>.

Acemannan, a major carbohydrate of polymannose fraction of *Aloe vera* gel, has been known to have antiviral and antitumoral activities *In vivo* through activation of immune responses. The antitumor activity of acemannan is thought to be mediated via activation of host defence mechanisms. Phenotypic analysis for the expression of class II MHC molecules and major co-stimulatory molecules such as B7-1, B7-2, CD40, and CD54 confirmed that acemannan could induce maturation of immature dendritic cells (DCs). Functional maturation of immature DCs was supported by increased allogenic mixed lymphocyte reaction and IL-12 production. The authors showed that acemannan could induce functional and phenotypic maturation of immature dendritic cells *In vitro*, and proposed the adjuvant activity of acemannan due to its capacity to promote differentiation of immune dendritic cells<sup>[10]</sup>.

The anti-inflammatory activity of *Aloe vera* was investigated through matrix metalloproteinase-9 (MMP-9) inhibition study. The effect of *Aloe vera* aqueous extract on MMP-9 inhibition was tested on peripheral blood mononuclear cells (PBMC), and *Aloe vera* aqueous extract indicated the *In vitro* inhibitory effect on MMP-9<sup>[11]</sup>.

Antitumor activity of 50% ethanol extract (100 mg/kg) of *Aloe vera* was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice. After 14 h of tumor inoculation, the extract was administered daily for 14 days. After administration of the last dose followed by 18 h fasting, mice were sacrificed for observation of antitumor activity. The effect of *Aloe vera* extract on the growth of transplantable ascites tumor, body weight of EAC bearing hosts and simultaneous alterations in the hematological profile, serum (ALT, AST, LDH, ALP and glucose) and liver biochemical parameters (lipid peroxidation, GSH and antioxidant enzymes) were estimated. The *Aloe vera* extract showed decrease in abdominal circumference and body weight of EAC tumor bearing mice. Hematological profile reverted towards normal levels in extract treated mice. Treatment with *Aloe vera* extract restored the serum biochemical parameters towards normal levels and decreased the levels of lipid peroxidation and increased the levels of reduced glutathione and other antioxidant enzymes (SOD, CAT, and GPX). The 50% ethanol extract of *Aloe vera* exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defence system in EAC bearing mice<sup>[12]</sup>.

In the study of *Aloe vera* leaf pulp extract against Ehrlich ascites tumors in mice, the animals were separated into five groups: 1-healthy control, 2-tumor control, 3-experiment 1, extract given before tumor inoculation, 4-experiment 2, extract given with tumor inoculation, and 5-experiment 3, extract given after tumor inoculation. Ehrlich ascites tumor, 0.33 mL, were injected subcutaneously into groups 2-5. Aloe extract was injected at 55 mg protein/kg, twice a week for 21

days. Tumor size, thymus and spleen weights were measured, as well as leucocyte count, tumor necrosis factor- $\alpha$  and sialic acid as tumor markers. The best inhibitory effect on tumor growth was obtained with the extract given prophylactically before tumor implantation, experiment 1, although aloe extract regressed tumor sizes when given simultaneously with experiment 2, or therapeutically after experiment 3, tumor implantation. Serum sialic acid and tumor necrosis factor- $\alpha$  levels, chosen as tumor markers, which were raised in the tumor control group, were significantly decreased by the prophylactic administration of the extract. The increase in leucocyte count seen in experiment 1 and 2 groups, along with lymphoid hyperplasia observed in spleen and thymus necroscopy, showed that the tumor preventive effect of aloe extract could be due to its immunomodulatory activity. Accordingly, the authors proposed aloe leaf pulp extract as a prophylactic for cancer prevention<sup>[13]</sup>.

The prophylactic effect of lectin in *Aloe vera* pulp extract (alocin 1) was assayed against Ehrlich ascites tumors in mice. Prophylactical administration of alocin 1 before tumor implantation, the tumor regressed the size. The authors showed that serum sialic acid and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels, chosen as tumor markers, were decreased significantly by the prophylactic administration of alocin 1. The increase in spleen and thymus weights in the group given only alocin 1, could be explained by immunomodulatory and mitogenic effects of alocin 1. These findings suggest that tumor preventive effect of alocin 1 could be due to its immunomodulatory activity<sup>[14]</sup>.

### Preliminary clinical study

Preliminary clinical study by the authors had shown that melatonin may induce some benefits in untreatable metastatic solid tumor patients, whereas, for the time being, no clinical trial has been performed with aloe products<sup>[15]</sup>. The authors have carried out a clinical study to evaluate whether the concomitant administration of *Aloe vera* may enhance the therapeutic results of melatonin in patients with advanced solid tumors for whom no effective standard anticancer therapies are available. The study included 50 patients suffering from lung cancer, gastrointestinal tract tumors, breast cancer or brain glioblastoma, who were treated with melatonin alone (20 mg/kg orally in the dark period) or melatonin plus *Aloe vera* tincture (1 mL twice/day). A partial response was achieved in 2/24 patients treated with melatonin plus aloe and in none of the patients treated with melatonin alone. Stable disease was achieved in 12/24 and in 7/26 patients treated with melatonin plus aloe or melatonin alone, respectively. Therefore, the percentage of non-progressing patients was significantly higher in the group treated with melatonin plus aloe than in the melatonin group (14/24 vs 7/26,  $p < 0.05$ ). The percent 1-year survival was significantly higher in patients treated with melatonin plus aloe (9/24 vs 4/26,  $p < 0.05$ ). Both treatments were well tolerated. This preliminary study would suggest that natural cancer therapy with melatonin plus *Aloe vera* extract may produce some therapeutic benefits, at least in terms of stabilization of disease and survival, in patients with advanced solid tumors, for whom no other standard effective therapy is available<sup>[16]</sup>.

The advances in the analysis of tumor immunobiology suggest the possibility of biological manipulating the efficacy and toxicity of cancer chemotherapy by endogenous or exogenous immunomodulating substances. Aloe is one of the most important plants exhibiting anticancer activity and its anti-neoplastic property is due to at least three different mechanisms, based on anti-proliferative, immuno-stimulatory and antioxidant effects. The anti-proliferative action is determined by anthracenic and anthraquinonic molecules,

while the immuno-stimulating activity is mainly due to acemannan. The authors started preliminary clinical aloe study as follows. **Patients and Method:** A study was planned to include 240 patients with metastatic solid tumor who were randomized to receive chemotherapy with or without aloe. According to tumor histotype and clinical status, lung cancer patients were treated with cisplatin and etoposide or weekly vinorelbine, colorectal cancer patients received oxaliplatin plus 5-fluorouracil, gastric cancer patients were treated with weekly 5-fluorouracil and pancreatic cancer patients received weekly gemcitabine. Aloe was given orally at 10 mL thrice/day. **Results:** The percentage of both objective tumors regression and disease control was significantly higher in patients concomitantly treated with aloe than with chemotherapy alone, as well as the percent of 3-year survival patients. **Conclusion:** This study seems to suggest that aloe may be successfully associated with chemotherapy to increase its efficacy in terms of both tumor regression rate and survival time<sup>[17]</sup>.

#### ***In vitro* and animal studies of aloin and aloe emodin**

The authors showed the cytotoxic activity of aloin against two human breast cancer cell lines; without MCF-7 and with SKBR-3 erbB-2-topoisomerase II $\alpha$  coamplification. MCF-7 cell line was shown to be more sensitive to aloin than SKBR-3 demonstrated by MTT and clonogenic assays, from which IC<sub>50</sub> and 50% ICF values are reported to be 60  $\mu$ g/mL, respectively, in the former cell line and as high as 150 and 80  $\mu$ g/mL, respectively, in the latter, which are still far below the maximum tolerated dose of the compound. The effect of aloin is suggested to be brought about by more than one mechanism depending on the dose level and tumor phenotype. This was demonstrated by flow cytometric analysis, fluorescence microscopy and western blot analysis, which revealed that aloin at high concentrations caused a reduction in the proportion of cells undergoing mitosis by induction of apoptosis, inhibition of topo II $\alpha$  protein expression and downregulation of cyclin B1 protein expression in MCF-7 cell line, whereas erbB-2 protein expression was not affected. Topo II $\alpha$  protein expression was mildly downregulated in SKBR-3 cell line at high concentration only<sup>[18]</sup>.

The authors have evaluated the chemopreventive efficacy of aloin against 1,2-dimethylhydrazine (DMH)-induced preneoplastic lesions in the colon of Wistar rats. DMH-induced aberrant crypt foci (ACF) and mucin-depleted foci (MDF) have been used as biomarkers of colon cancer. Efficacy of aloin against the colon toxicity was evaluated in terms of biochemical estimation of antioxidant enzyme activities, lipid peroxidation, ACF, MDF, histopathological changes, and expression levels of molecular markers of inflammation and tumor promotion. Aloin pretreatment ameliorates the damaging effects induced by DMH through a protective mechanism that involved reduction in increased oxidative stress enzymes ( $p < 0.001$ ), ACF, MDF, cyclooxygenase-2, inducible nitric oxide synthase, interleukin-6, proliferating cell nuclear antigen protein expression, and tumor necrosis factor- $\alpha$  ( $p < 0.001$ ) release. From the results, it could be concluded that aloin clearly protects against chemically induced colon toxicity and acts reasonably by inducing antioxidant level, anti-inflammatory and antiproliferative markers<sup>[19]</sup>.

Angiogenesis has been an attractive target for drug therapy. Aloin has been to possess anti-cancer potential activities. However, its roles in tumor angiogenesis and the involved molecular mechanism are unknown. **Method:** To evaluate the angiogenic and anticancer activities of aloin, endothelial cell scratch, modified Boyden chamber inserts and tube formation assays were done in human umbilical vein endothelial cells (HUVECs), and MTT and Live-Dead assays were used to determine the proliferation inhibition and apoptosis

induction of colorectal cancer cells *In vitro*. The inhibition effects of aloin were further confirmed by a mouse xenograft model *In vivo*. The expression levels of signal transducer and activator of transcription 3 (STAT3) signaling pathway and that mediated-target genes were measured in HUVECs and SW620 cells by Western blots. **Results:** Aloin significantly inhibited HUVECs proliferation, migration and tube formation *In vitro*. Western blotting showed that aloin suppressed activation of VEGF receptor 2 and STAT3 phosphorylation in endothelial cells. In addition, the constitutively activated STAT3 protein, and the expression of STAT3-regulated antiapoptotic (Bcl-xL), proliferative (c-Myc), and angiogenic (VEGF) proteins were also down-regulated in response to aloin in human SW620 cancer cells. Consistent with the above findings, aloin inhibited tumor cell viability and induced cell apoptosis *In vitro*, and substantially reduced tumor volumes and weight *In vivo* mouse xenografts, without obviously toxicity. **Conclusion:** Present studies provided the first evidence that aloin may inhibit tumor angiogenesis and growth via blocking STAT3 activation, with the potential of a drug candidate for cancer therapy<sup>[20]</sup>.

The authors demonstrated that aloin exerted inhibition of cell proliferation, adhesion and invasion abilities of B16-F10 melanoma cells under non-cytotoxic concentrations. Furthermore aloin induced melanoma cell differentiation through the enhancement of melanogenesis and transglutaminase activity. To improve the growth-inhibiting effect of anticancer agents, the authors found that the combined treatment of cells with aloin and low doses of cisplatin increases the antiproliferative activity of aloin. The results suggest that aloin possesses antineoplastic and antimetastatic properties, exerted likely through the induction of melanoma cell differentiation<sup>[21]</sup>.

The authors reported that aloe emodin has a specific *In vitro* and *In vivo* antineuroectodermal tumor activity. The growth of human neuroectodermal tumors is inhibited in mice with severe combined immunodeficiency without any appreciable toxic effects on animals. The compound does not inhibit the proliferation of normal fibroblasts nor that of hemopoietic progenitor cells. The cytotoxicity mechanism consists of the induction of apoptosis, whereas the selectivity against neuroectodermal tumor cells is found on a specific energy-dependent pathway of drug incorporation. Taking into account its unique cytotoxicity profile and mode of action, aloe emodin might represent a conceptually new lead antitumor drug<sup>[22]</sup>.

The authors attempted to clarify the intracellular target of aloe emodin and the apoptosis-signaling pathway activated by aloe emodin in neuroblastoma cell lines. Two-photon excitation microscopy and spectroscopic titrations documented that aloe emodin is a highly concentrated in susceptible cells and binds to DNA. One of the most important mediators of apoptotic response to genotoxic stimuli, such as anticancer agents, is the p53 tumor suppressor gene. To evaluate the role played by p53 in aloe emodin-induced apoptosis a p53 mutant cell line, which lacks transcriptional activity of p53 targeted gene, was tested. Aloe emodin displayed a reduced growth inhibitory and pro-apoptotic activity in p53 mutant cells [SK-N-BE(2c)] with respect to the p53 wild-type line (SJ-N-KP). This effect was not caused by a reduced drug uptake in the mutual neuroblastoma cell line but was related to a different apoptotic cell phenotype. Whereas SJ-N-KP cells were susceptible to a p53 transcription-dependent pathway of apoptosis, SK-N-BE(2c) cells underwent apoptosis with up-regulation of p53 expression but not of p53-target genes. After aloe emodin treatment p53 translocates to the mitochondria inter-membrane space in both neuroblastoma cell lines. Due to its high accumulation in neuroectodermal tumor cells aloe

emodin could also kill tumor cells harboring p53 mutant genes. This property would further contribute to aloe emodin specific anti-tumor activity and might be exploitable in the clinic<sup>[23]</sup>.

The authors identified aloe emodin as a new anti-angiogenic compound with inhibitory effects in an *In vivo* angiogenesis assay and evaluated its effects on specific key steps of the angiogenic process. Aloe emodin inhibits endothelial cell proliferation, but this effect is not cell specific, since aloe emodin also inhibits tumor cell proliferation. Cell migration and invasion are not remarkably affected by aloe emodin. On the other hand, aloe emodin has different effects on endothelial and tumor cell gelatinases. Two main targets of the pharmacological action of aloe emodin as an anti-angiogenic compound seem to be urokinase secretion and tube formation of endothelial cells. Finally, aloe emodin produces a remarkable photocytotoxic effect on tumor cells. Taken together, the present data indicate that aloe emodin can behave both as an anti-tumor and an anti-angiogenic compound and suggest that aloe emodin could be a candidate drug for photodynamic therapy<sup>[24]</sup>.

The authors demonstrated the capacity of aloe emodin to reduce the cytotoxicity of the proinflammatory cytokine tumor necrosis factor (TNF) towards L929 mouse fibrosarcoma and U251 human glioma cell lines. Aloe emodin inhibited both TNF-induced cell necrosis and apoptosis, but it did not reduce cell death induced by UV radiation or hydrogen peroxide. Aloe emodin inhibited both basal and TNF-triggered activation of extracellular signal-regulated kinase (ERK), and a selective blockade of ERK activation mimicked the cytoprotective action of the drug. On the other hand, aloe emodin did not affect TNF-induced activation of p38 mitogen-activated protein kinase or generation of reactive oxygen species. The combination of aloe emodin and TNF caused an intracellular appearance of acidified autophagic vesicles, and the inhibition of autophagy with bafilomycin or 3-methyladenine efficiently blocked the cytoprotective action of aloe emodin. These data indicate that aloe emodin could prevent TNF-triggered cell death through mechanisms involving induction of autophagy and blockade of ERK activation<sup>[25]</sup>.

The authors investigated the anticancer effect of aloe emodin on two distinct human gastric carcinoma cell lines, AGS and NCI-N87. The authors demonstrated that aloe emodin induced cell death in a dose- and time-dependent manner. Noteworthy is that the AGS cells were generally more sensitive than the NCI-N87 cells. Aloe emodin caused the release of apoptosis-inducing factor and cytochrome c from mitochondria, followed by the activation of caspase-3, leading to nuclear shrinkage and apoptosis. In addition, exposure to aloe emodin suppressed the casein kinase II activation in a time-dependent manner and was accompanied by a reduced phosphorylation of Bid, a downstream substrate of casein kinase II and a pro-apoptotic molecule. These preliminary clinical studies suggest that aloe emodin represents a suitable and novel chemotherapeutic drug candidate for the treatment of human gastric carcinoma<sup>[26]</sup>.

The authors studied the antineoplastic properties of aloe emodin highly metastatic B16-F10 melanoma murine cells. **Main methods:** Cell proliferation was assessed by counting and viability was investigated using MTT and Trypan Blue exclusion tests. As a growth marker, the authors determined intracellular polyamine levels by high performance liquid chromatography. Then, the authors evaluated transglutaminase 2 (TG2) activity, protoporphyrin IX accumulation and melanin content as differentiative markers. Tyrosinase activity was checked by DOPA-staining assay. The antimetastatic effect of aloe emodin was evaluated by means of a series of *In vitro* metastatic assays, including aggregation, wound healing migration, adhesion, 3D-inversion, circular invasion and

the Boyden chamber invasion assays. Gelatin zymography was performed to evaluate metalloproteinase activities. **Key findings:** The results demonstrated inhibitory effects of aloe emodin on melanoma cell proliferation and invasion power, accompanied by the stimulation of cell differentiation parameters. Cell differentiation correlated with a remarkable increase of the capacity of the transamidating form of TG2, with a significant enhancement of cell adhesion and aggregation. Impaired invasion was paralleled by the decrease of the secretion of matrix metalloproteinase-9. **Significance:** The overall data confirm a remarkable antiproliferative, antimetastatic and differentiative capability of aloe emodin. Results suggest that aloe emodin appears particularly promising for its potential application in the newborn differentiation therapy of cancer<sup>[27]</sup>.

The authors investigated the possible modulation of defined markers of monocytic differentiation of aloe emodin on human U937 cell line. **Main methods:** U937 cells differentiation has been confirmed unequivocally by Griss and nitroblue tetrazolium reduction assays, protoporphyrin IX accumulation, expression of CD14 and CD11b surface antigens, phagocytic activity, migration and attachment ability. The effect on polyamine metabolism, apoptosis and cytokine production was also investigated. **Key findings:** Aloe emodin-treated U937 cells exhibit a noticeably rise in transglutaminase activity. This enhanced enzyme activity correlates with aloe emodin-induced growth arrest and differentiation to functionally mature monocytes. **Significance:** Taken together, the results reported that aloe emodin can promote the macrophage differentiation of U937 cells, suggesting that aloe emodin could be a potential candidate as a differentiation-inducing selective agent for therapeutic treatment of leukemia<sup>[28]</sup>.

The mechanisms of aloe emodin anti-cancer effect are largely unknown. The authors investigated its molecular mechanisms. Crystal violet assay showed that aloe emodin had a long-term anti-proliferation effect on human gastric cancer MGC-803 and SGC-7901 cells. Scratch wound-healing motility assays indicated its anti-migration effect. Aloe emodin arrested SGC-7901 cells at G2/M phase. More importantly, aloe emodin inhibited the expressions of protein kinase C and c-myc. In conclusion, the anti-cancer effect of aloe emodin on gastric cancer cells involves suppression of c-myc expression<sup>[29]</sup>.

Colon carcinoma cells were treated with various concentrations of aloe emodin for different durations. Cell viability was measured by sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate assay. DNA fragmentation was analyzed by agarose gel electrophoresis. Nuclear shrinkage was visualized by Hoechst 33,258 staining. Western blotting was used to indicate the release of apoptosis-inducing factor and cytochrome c from mitochondria and the phosphorylation of Bid. Caspase-3 and casein kinase II activities were measured by the respective assays. Cell viability analyses showed that aloe emodin induced cell death in a dose- and time-dependent manner. Notably, the WiDr cells were more sensitive to aloe emodin than the DLD-1 cells. Aloe emodin caused the release of apoptosis-inducing factor and cytochrome c from mitochondria, followed by activation of caspase-3 leading to DNA fragmentation, nuclear shrinkage and apoptosis. In addition, exposure of colon carcinoma cells to aloe emodin suppressed the casein kinase II activity in a time-dependent manner and was accompanied by a reduced phosphorylation of Bid, a downstream substrate of casein kinase II activity, the release of apoptosis-inducing factor and cytochrome c, and the caspase-3 activation are involved in aloe emodin-mediated apoptosis in colon carcinoma cells<sup>[30]</sup>.

The authors analyzed molecular mechanisms involved in the antimigratory and antiangiogenic activity of aloe emodin in colon cancer cell, WiDr. The results show that a relatively non toxic concentration of aloe emodin suppressed the phorbol-12-myristyl-13-acetate induced migration and invasion of tumor cells. On analysis for the molecules involved in the migration/invasion, the authors found aloe emodin downregulated mRNA expression and promoter/gelatinolytic activity of matrix metalloproteinase (MMP)-2/9, as well as the RhoB expression at gene and protein level. Aloe emodin was also a strong inhibitor of vascular endothelial growth factor (VEGF) expression, promoter activity and endothelial cell migration/invasion and *In vitro* angiogenesis. Aloe emodin suppressed the nuclear translocation and DNA binding of NF- $\kappa$ B, which is an important transcription factor for controlling MMP-2/9 and VEGF gene expression. Taken together, these data indicate that aloe emodin targets multiple molecules responsible for cellular invasion, migration and angiogenesis. Inhibitory effect on angiogenic and metastatic regulatory processes make aloe emodin a sensible candidate as a specific blocker of tumor associated events<sup>[31]</sup>.

Phosphatidylinositol 3-kinase (PI3-K) amplification and phosphatase and tensin homolog (PTEN) deletion-caused Akt activation contribute to the development of prostate cancer. Mammalian target of rapamycin complex 2 (mTORC2) is a kinase complex comprised of mTOR, Rictor, mSin1, mLST8/G $\beta$ L and PRR5 and functions in the phosphorylation of Akt at Ser473. The authors reported that mTORC2 plays an important role in PC3 androgen refractory prostate cell proliferation and anchorage-independent growth. Aloe emodin inhibited both proliferation and anchorage-independent growth of PC3 cells. Protein content analysis suggested that activation of downstream substrates of mTORC2, Akt and PKC  $\alpha$ , was inhibited by aloe emodin treatment. Pull-down assay and *In vitro* kinase assay results indicated that aloe emodin could bind with mTORC2 in cells and inhibit its kinase activity. Aloe emodin also exhibited tumor suppression effects *In vivo* in an athymic nude mouse model. Collectively, the present data suggest that mTORC2 plays an important role in prostate cancer development and aloe emodin suppresses prostate cancer progression by targeting mTORC2<sup>[32]</sup>.

To determine how aloe emodin regulates the cell cycle, cell proliferation and protein kinase C (PKC) during glioma growth and development, the authors established the cell cycle effects of aloe emodin on brain cells [transformed glia cell line (SVG) and human glioma U-373MG cell line (U-373MG)], were treated with either dimethylsulfoxide (DMSO; control) or aloe emodin (40  $\mu$ M). Results from flow cytometry demonstrated that aloe emodin delayed the number of cells entering and exiting DNA synthesis (S) phase in both SVG and U-373MG cells indicating that aloe emodin may inhibit S phase progression. Assessment of cell viability demonstrated that SVG and U-373MG glioma cell were highly sensitive to aloe emodin. The aloe emodin-induced decreased proliferation was sustained at 48-96 h. A PKC activity assay was quantified to establish the role of PKC in aloe emodin's mode of action. Exposure of SVG and U-373MG glioma cells to aloe emodin suppressed PKC activity and reduced the protein content of most of the PKC isozymes. The authors determined that cancer growth inhibition by aloe emodin was due to apoptosis. Taken together, these results support the hypothesis that aloe emodin represents a novel antitumor chemotherapeutic drug<sup>[33]</sup>.

The authors investigated the effect of aloe emodin on the rat C6 glioma cell line. In addition to cell cycle block and caspase dependent apoptosis, aloe emodin led to the formation of intracytoplasmic acidic vesicles inductive for autophagic cell death.

Moreover, differentiation of surviving cells toward the astrocytic lineage was confirmed by typical morphological changes and increased expression of glial fibrillary acidic protein (GFAP). Aloe emodin did not affect the activation of mitogen-activated protein kinase p38, Jun-N-terminal kinase, or transcription factor NF- $\kappa$ B, but markedly inhibited the activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) in C6 cells. A selective inhibitor of ERK activation, PD98059, mimicked the effects of aloe emodin on glioma cell morphology and GFAP expression, but failed to induce either apoptosis or autophagy. Taken together, these results indicated that the anti-glioma action of aloe emodin involves ERK-independent induction of both apoptosis and autophagy, as well as ERK inhibition-mediated differentiation of glioma cells<sup>[34]</sup>.

The authors investigated the apoptosis and cell cycle arrest inducing by aloe emodin on U87 human malignant glioma cells. Aloe emodin showed a time- and dose- dependent inhibition of U87 cells proliferation and decreased the percentage of variable U87 cells via the induction of apoptosis. Characteristic morphological changes, such as the formation of apoptotic bodies, were observed with confocal microscope by Annexin V-FITC/PI staining, supporting the viability study and flow cytometry analysis results. The present data demonstrated that aloe emodin arrested the cell cycle in the S phase and promoted the loss of mitochondrial membrane potential in U87 cells that indicated the early event of the mitochondria-induced apoptosis pathway<sup>[35]</sup>.

The authors investigated the effects of aloe emodin on the growth of human cervical cancer cells, HeLa. **Methods:** HeLa cells were treated with various concentrations of aloe emodin for 1-5 d, and cell growth was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. The long-term growth effect was investigated by crystal violet assay. The distributions of the cell cycles and apoptosis were analyzed by flow cytometry. The alkaline phosphatase (ALP) activity was analyzed by a chemical analyzer. Finally, Western blotting was used to indicate the abundant changes of protein kinase C (PKC), c-myc, cyclins, cyclin-dependent kinases (CDK), and proliferating cell nuclear antigen (PCNA). **Results:** Aloe emodin inhibited the growth of HeLa cells in a dose-dependent manner at concentrations ranging between 2.5 and 40  $\mu$ M/L. The flow cytometric analysis showed that HeLa cells were arrested at the G2/M phase. This effect was associated with the decrease in cyclin A and CDK2, and the increase in cyclin B1 and CDK1. More importantly, the ALP activity was found to be increased by aloe emodin treatment, and accompanied by the inhibition of PCNA expression. In addition, aloe emodin suppressed the expression of PKC and c-myc. **Conclusion:** These findings provide a possible mechanistic explanation for the growth inhibitory effect of aloe emodin on HeLa, which includes cell cycle arrest and inducing differentiation<sup>[36]</sup>.

The purpose of the study was to investigate the anti-cancer effect of aloe emodin on human tongue squamous carcinoma SCC-4 cells. The results indicated that aloe emodin induced cell death through S-phase arrest and apoptosis in a dose- and time-dependent manner. Treatment with 30  $\mu$ M of aloe emodin led to S-phase arrest through promoted p53, p21 and p27, but inhibited cyclin A, E, thymidylate synthase and Cdc25A levels. Aloe emodin promoted the release of apoptosis-inducing factor, endonuclease G, pro-caspase-9 and cytochrome c from the mitochondria via a loss of the mitochondrial membrane potential which was associated with a increase in the ratio of B-cell lymphoma 2-associated  $\times$  protein (Bax)/B cell lymphoma/leukemia-2 (Bcl-2) and activation of caspase-9 and -3. The free radical scavenger N-acetylcystein and caspase inhibitors markedly blocked aloe emodin-induced apoptosis. Aloe emodin thus

induced apoptosis in the SCC-4 cells through the Fas/death-receptor, mitochondria and caspase cascade. Aloe emodin could be a novel chemotherapeutic drug candidate for the treatment of human tongue squamous cancer in the future<sup>[37]</sup>.

Aloe emodin has been shown to induce apoptosis in several cancer cell lines *In vitro*. However, its molecular mechanism of action in the apoptosis induction of human nasopharyngeal carcinoma (NPC) cells has not been explored. This study shows that aloe emodin induced G(2)/M phase arrest by increasing levels of cyclin B1 bound to Cdc2, and also caused an increase in apoptosis of NPC cells, which was characterized by morphological changes, nuclear condensation, DNA fragmentation, caspase-3 activation, cleavage of poly (ADP-ribose) polymerase and increased sub-G<sup>[1]</sup> population. Treatment of NPC cells with aloe emodin also resulted in a decrease in Bcl-X(L) and an increase in Bax expression. Ectopic expression of Bcl-X(L) but not Bcl-2 or small interfering RNA-mediated attenuation of Bax suppressed aloe emodin-induced apoptotic cell death. Aloe emodin-induced loss of mitochondrial membrane potential and increase in cellular Ca<sup>2+</sup> content, reactive oxygen species and apoptotic cell death were suppressed by the treatment of cyclosporin A or caspase-8 inhibitor. Co-treatment with caspase-9 inhibitor Z-LEHD-FMK could inhibit aloe emodin-induced cell death and the activation of caspase-3 and -9. In addition, suppression of caspase-8 with the specific inhibitor Z-IETD-FMK inhibited aloe emodin-induced the activation of Bax, the cleavage of Bid, the translocation of tBid to the mitochondria and the release of cytochrome c, apoptosis-inducing factor and Endo G from the mitochondria and subsequent apoptosis. Taken together, these results indicate that the caspase-8-mediated activation of the mitochondrial death pathway plays a critical role in aloe emodin-induced apoptosis of NPC cell<sup>[38]</sup>.

Using short hairpin RNA against p53, transist ectopic expression of wild-type p53 or mutant p53 (R248W or R175H), and a p53- and p21-dependent luciferase reporter assay, the authors demonstrated that growth arrest and apoptosis of FaDu (human pharyngeal squamous cell sarcoma), Hep3B (hepatoma), and MG-63 (osteosarcoma) cells induced by aloe emodin are p53-independent. Co-immuno-precipitation and small interfering RNA (siRNA) studies demonstrated that aloe emodin caused S-phase cell cycle arrest by inducing the formation of cyclinA-Cdk2-p21 complexes through extracellular signal-regulated kinase (ERK) activation. Ectopic expression of Bcl-X(L) and siRNA-mediated Bax attenuation significantly inhibited apoptosis induced by aloe emodin. Cyclosporin A or the caspase-8 inhibitor Z-IETD-FMK blocked aloe emodin-induced loss of mitochondrial membrane potential and prevented increases in reactive oxygen species and Ca<sup>2+</sup>. Z-IETD-FMK inhibited aloe emodin-induced apoptosis, Bax expression, Bid cleavage, translocation of tBid to mitochondria, ERK phosphorylation, caspase-9 activation, and the release of cytochrome c, apoptosis-inducing factor, and endonuclease G from mitochondria. The stability of the mRNAs encoding caspase-8 and -10-associated RING proteins (CARPs) 1 and 2 was affected by aloe emodin, whereas CARP1 or 2 overexpression inhibited caspase-8 activation and apoptosis induced by aloe emodin. Collectively, the present data indicate aloe emodin induces caspase-8-mediated activation of mitochondrial death pathways by decreasing the stability of CARP mRNAs in a p53-independent manner<sup>[39]</sup>.

To evaluate the potential anticancer properties and modulatory effect of selected *Aloe vera* three active principles on antioxidant enzyme activities; aloesin, aloe emodin and barbaloin were extracted from *Aloe vera* leaves by supercritical fluid extraction and subsequently purified by high performance liquid chromatography.

Additionally, the N-terminal octapeptide (DEDNVLLT) moiety from verectin, an anti-inflammatory glycoprotein (Mw:14KDa) present in *Aloe vera* gel, was also tested. *In vivo*, active principles exhibited significant prolongation of the life span of tumor-transplanted animal in the following order: barbaloin>octapeptide>aloesin>aloe emodin. *Aloe vera* active principles exhibited significant inhibition on Ehrlich ascites carcinoma cell (EACC) number, when compared to positive control group, in the following order: barbaloin>aloe emodin>octapeptide>aloesin. Moreover, in trypan blue cell viability assay, active principles showed a significant concentration-dependent cytotoxicity against acute myeloid leukemia (AML) and acute lymphocytes leukemia (ALL) cancerous cells. Furthermore, in MTT cell viability test, aloe emodin was found to be active against two human colon cancer cell lines (i.e. DLD-1 and HT2), with IC50 values of 8.94 and 10.78  $\mu$ M, respectively. Treatments of human AML leukemic cells with active principles (100  $\mu$ g/ml) resulted in varying intensities of inter-nucleosomal DNA fragmentation, hallmark of cells undergoing apoptosis, in the following order: aloe emodin >aloesin>barbaloin>octapeptide. Interestingly, treatment of EACC tumors with active principles resulted in a significant elevation activity of key antioxidant enzymes (SOD, GST, tGPx, and LDH). The present data suggest that the tested *Aloe vera* compounds may exert their chemo-preventive effect through modulating antioxidant and detoxification enzyme activity levels, as they are one of the indicators of tumorigenesis. These findings were discussed in the light of the potential of *Aloe vera* plant extracts for developing efficient specific and non-toxic anticancer drugs<sup>[40]</sup>.

#### Alleviation of progeria

The Hutchinson Gilford progeria syndrome is a premature aging disorder in children. Progeria is a cause of progerin accumulation due to mutation in protein lamin A which can be inhibited by blocking the prenylation process. The addition of a farnesyl or geranyl moiety to the CAAX motif of the protein in the post translation step prevents the formation of mutant lamin A. In the present study, the inhibitors were screened for blocking the prenylation process by docking nutraceuticals with the farnesyl pyrophosphate synthase (FPPS) using Autodock. Aloe emodin showed binding energy (-8.12 Kcal/mol), inhibitory constant (1.11  $\mu$ M) forming 6 hydrogen bond interactions with the active site residues Lys257, Arg112, Try204, Lys200, Thr201 and Arg112 of FPPS target<sup>[41]</sup>.

## PREVENTION OF CARDIOVASCULAR AND CEREBRO-VASCULAR DISORDERS, AND INHIBITION OF ACETYLCHOLINESTERASE

#### Prevention of cardiovascular disorder

The cardio-excitatory effect of aloe extract on the isolated atria was demonstrated and the chemical and spectral examinations led to the conclusion that compounds I-IV, isolated from aloe saponaria leaf pulp extract, using solvent partition, nonionic porous resin, and gel permeation chromatographies, are calcium salts of (+)-isocitric, L(-)-malic, succinic, and of (-)-2-hydroxybutandiolic acid 4-methyl ester, respectively. Cardiac stimulant activity of calcium (+)-isocitrate isolated and synthesized stereoisomers of calcium isocitrate was demonstrated and a positive inotropic effect showed in isolated guinea pig atria at a concentration of 10<sup>-4</sup> g/ml, only in the form of calcium salts, while no toxic effect such as arrhythmia was observed<sup>[42]</sup>.

Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is a nona-

peptide that cause blood vessels to dilate (enlarge), and therefore causes blood pressure to lower; vasodilation of arteries; vein of gut, and stimulate pain receptors. In human, bradykinin is broken down by three proteolytic kinases; angiotensin-converting enzyme, amino peptidase, and carboxypeptidase N, which cleave the 7-8, 1-2, and 8-9 positions, respectively. A homogeneous aloe glycoprotein (mol. mass 40,000 D) containing 50.7 % of protein was isolated from an extract of *Aloe arborescens var natalensis* by precipitation with 60% ammonium sulfate. The aloe glycoprotein had bradykinin-degrading activity on an isolated guinea pig ileum *In vitro*. Peptide analysis using a reversed-phase, high performance liquid chromatography coupled with amino acid analysis showed that aloe glycoprotein cleaves the Pro<sup>7</sup>-Phe<sup>8</sup> and Phe<sup>8</sup>-Arg<sup>9</sup> bonds of the bradykinin molecule. The proteolytic action suggests that aloe glycoprotein has carboxypeptidase N- and P-like activity<sup>[43]</sup>.

The authors tested bradykinin-induced contraction of the isolated rat ileum with *Aloe vera* gel and found the presence of a material that inhibits the bradykinin effect, which might explain the anti-inflammatory properties of *Aloe vera* gel<sup>[44]</sup>.

The authors presented the effect of *Aloe vera* gel on contractile and other property of rat myocardium using isolated heart model. **Methods:** A total of 24 male albino rats were divided into four groups, one control and three experimental of six animals each. *Aloe vera* gel extract was prepared by taking 10 g of gel, dissolved in distilled water on vortex for 15 min, and filtered the solution. The hearts were perfused with aqueous solutions of aloe in doses of 100 mg, 200 mg, and 300 mg/L of Kreb's solutions. **Observation and results:** The results showed that there were no significant difference in baseline heart rate (beats per min) and force of contraction (in mm) among control and experimental groups. *Aloe vera* in dose of 200 mg/L and 300 mg/L of Kreb's-Henseleits solution was intervened showed highly significant change in force of contraction and coronary flow as compared in control. **Conclusion:** The present study showed that *Aloe vera* causes increase in myocardial force of contraction and coronary flow; therefore *Aloe vera* may be cardio-protective and will be helpful in re-establishing the ischemic myocardium<sup>[45]</sup>.

Diabetes is a metabolic syndrome characterized by hyperglycemia, hypercholesterolemia and hypertriglyceridemia. Hence, there is a need to search the anti-diabetic drugs which apart from lowering the blood glucose levels can also modify the atherogenic lipid profile without producing many side effects. Oral administration of *Aloe vera* leaf extract for 21 days in alloxan induced diabetic rabbits produced a significant reduction in fasting blood glucose levels and HbA<sub>1c</sub>. Also there was significant decrease in serum levels of triglyceride, total cholesterol, lower density lipoprotein cholesterol and a concomitant increase in high density lipoprotein cholesterol in *Aloe vera* treated diabetic rabbit indicates the potential of *Aloe vera* as anti-diabetic drug. The significant decrease in atherogenic index in *Aloe vera* treated group showed its protection against cardio-vascular diseases<sup>[46]</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 seeds) and *Aloe vera*' to 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 nitrites, were tapered. The patients, most benefited, were diabetics (without adding any anti-diabetic 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>[47]</sup>.

#### Prevention of cerebro-vascular disorder

The central nervous system regulates systemic inflammatory responses to endotoxin (lipopolysaccharide) through humoral mechanisms. Activation of afferent vagus nerve fibres by endotoxin or cytokines stimulates hypothalamic-pituitary-adrenal anti-inflammatory responses. However, comparatively little is known about the role of efferent vagus nerve signalling in modulating inflammation. The authors describe a previously unrecognized, parasympathetic anti-inflammatory pathway by which the brain modulates systemic inflammatory responses to endotoxin. Acetylcholine, the principle vagal neurotransmitter, significantly attenuated the release of cytokines (tumor necrosis factor: TNF), interleukin (IL)-1 $\beta$ , IL-6 and IL-8, but not the anti-inflammatory cytokine IL-10, in lipopolysaccharide-stimulated human macrophage cultures. Direct electrical stimulation of the peripheral vagus nerve *In vivo* during lethal endotoxaemia in rats inhibited TNF synthesis in liver, attenuated peak serum TNF amounts, and prevented the development of shock<sup>[48]</sup>.

#### Inhibition of acetylcholinesterase

The author briefly commented about acetylcholinesterase inhibitors for Alzheimer's disease as possible anti-inflammatory agents through a "cholinergic anti-inflammatory pathway", as following. The pathogenesis of Alzheimer's disease (AD) has been linked to a deficiency in the brain neurotransmitter acetylcholine. Subsequently, acetylcholinesterase inhibitors (AChEIs) were introduced for the symptomatic treatment of AD. The prevailing view has been that the efficacy of AChEIs is attained through their augmentation of acetylcholine-mediated neuron to neuron transmission. However, AChEIs also protect cells from free radical toxicity and  $\beta$ -amyloid-induced injury, and increased production of antioxidants. In addition, it has been reported that AChEIs directly inhibit the release of cytokines from microglia and monocytes. These observations are supported by evidence showing a role for acetylcholine in suppression of cytokine release through a "cholinergic anti-inflammatory pathway". Based on the accumulating research data so far, it is no longer appropriate to consider that the sole action of AChEIs in AD is through direct acetylcholine-mediated enhancement of neuronal transmission. Evidence points to a possible anti-inflammatory role for these agents as well<sup>[49]</sup>.

The authors aimed to determine the spectrum of activity of *Aloe vera* gel on CNS. Effect of *Aloe vera* gel was studied on sodium nitrite induced hypoxia on elevated plus maze with pyritinol as positive control. *Aloe vera* gel attenuates the sodium nitrite induced memory impairment ( $p < 0.001$ ). To study the effect of *Aloe vera* gel peripheral cholinergic system, concentration response curve of acetylcholine (ACh) was plotted in presence and absence of *Aloe vera* gel. Dose ratio of ACh with *Aloe vera* gel (1 and 2 mg/mL) was found to be 0.846 and 0.692, respectively. This shows muscarinic receptor sensitizing effect of *Aloe vera* gel. Moreover, sodium nitrite-induced elevation of brain acetylcholinesterase (AChE) activity was significantly ( $P < 0.05$ ) lowered by pyritinol (100 mg/kg, p.s.) and *Aloe vera* gel (100 and 200 mg/kg, p.o.), indicating the counteracting action on the cholinergic system. Attenuation of sodium nitrite induced memory impairment and cholinergic muscarinic receptors sensitization suggest the role of *Aloe vera* gel for inflammatory

memory disorders like Alzheimer's<sup>[50]</sup>.

The authors investigated the effects of the *Aloe vera* aqueous extract and proteinous fractions of *Aloe vera* leaves on cholesterol, acetylcholinesterase in brain, glycogen, glutathione in liver and malonaldehyde levels in heart in normal male albino mice. The antioxidant properties and inhibition of acetylcholinesterase in tissue were detected. Intraperitoneal administration of *Aloe vera* extract in concentration of 400 mg/kg, significantly decreased the levels of AchE in brain by (-88.27%) and glutathione content in liver by (-35.48%), and increased the levels of glycogen in liver and malonaldehyde in heart by (22.60%, 85.50%), respectively. At a concentration of 300 mg/kg *Aloe vera* extract significantly increased the level of cholesterol in brain by (24.39%). These results clearly show the antioxidant property of the extract of *Aloe vera* leaves<sup>[51]</sup>.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are the key enzymes in pathogenesis of AD, which is characterized by a deficit in central cholinergic transmission. The authors investigated AChE and BChE inhibitory activities of seven coumarin derivatives obtained from *Hedercleum crenatifolium*, as well as of two anthraquinone derivatives and one stilbene, were tested by the spectrophotometric method of Ellman using an ELISA microplate-reader, at 1 mg/mL. Among them, the furanocoumarin mixture (68.8±0.76)%, bergapten (62.4±0.74)%, aloe emodin (57.2±1.32)%, scopoletin (53.1±0.83)%, and 4-methylumbelliferone (67.1±0.98)%, as well as the furanocoumarin mixture (76.7±0.95)% had a notable anti-BChE effect<sup>[52]</sup>.

The authors isolated scopoletin inhibiting the enzymatic activity of 5-lipoxygenase and acetylcholinesterase with an IC<sub>50</sub> equal to 1.76±0.01 μM and 0.27±0.02 mM, respectively, from *Canarium patentinervium*, and confronted oxidation in the ABT, DPPH, FRAP, and β-carotene bleaching assay with EC<sub>50</sub> values equal to 5.62±0.03 μM, 0.19±0.01 mM, 0.25±0.03 mM and 0.65±0.07 mM, respectively. Given the afore mentioned evidence, it is tempting to speculate that scopoletin represents an exciting scaffold from which to develop leads for treatment of neurodegenerative diseases<sup>[53]</sup>.

Alzheimer's disease (AD) is the most common neurodegenerative disorder to date. Neuropathological hallmarks are β-amyloid (Aβ) plaques and neurofibrillary tangles, but the inflammatory process has a fundamental role in the pathogenesis of AD. Inflammatory components related to AD neuroinflammation include brain cells such as microglia and astrocytes, the complement system, as well as cytokines and chemokines. Cytokines play a key role in inflammatory and anti-inflammatory processes in AD.

The authors showed that an important factor in the onset of inflammatory process is the overexpression of interleukin (IL)-1, which produces many reactions in vicious circle that cause dysfunction and neuronal death. Other important cytokines in neuroinflammation are IL-6 and tumor necrosis factor (TNF)-α. By contrast, other cytokines such as IL-1 receptor antagonist (IL-1ra), IL-4, IL-10, and transforming growth factor (TNF)-β can suppress both proinflammatory cytokine production and their action, subsequently protecting the brain. It has been observed in epidemiological studies that treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) decreases the risk for developing AD. Unfortunately, clinical trials of NSAIDs in AD patients have not been very fruitful. Proinflammatory responses may be countered through polyphenols. Supplementation of these natural compounds may provide a new therapeutic line of approach to this brain disorder<sup>[54]</sup>.

The authors designed, synthesized, and evaluated a series of aloe emodin derivatives as acetylcholinesterase inhibitors. Most of the new prepared compounds showed remarkable acetylcholinesterase (AChE)

inhibitory activities. Among them, the compound 1-(4,5-dihydroxy-9,10-dioxo-dihydroanthracen-2-yl) methylpyridin-1-ium chloride (C3) which has a pyridinium substituent in aloe emodin molecule, possessed the best inhibitory activity of acetylcholinesterase (IC<sub>50</sub>=0.09 μM). The docking study performed with AUTODOCK demonstrated that C3 could interact with the catalytic active site (CAS) and the peripheral anionic site (PAS) of acetylcholinesterase. The novel aloe emodin derivative (C3) which could interact with both the CAS and PAS of AChE, possessed the better AChE-inhibition activity than tacrine (IC<sub>50</sub>=0.09 μM, the IC<sub>50</sub> of tacrine is 0.26)<sup>[55]</sup>.

## PRELIMINARY CLINICAL STUDY OF AN ALOE POLYMANNOSE MULTINUTRIENT COMPLEX ON COGNITIVE AND IMMUNE FUNCTIONING IN ALZHEIMER'S DISEASE

### *In vitro* and animal studies

The authors had found that aloe emodin sulfates/glucuronides metabolites exerted a neuroprotective activity upon the retina ganglion cells (RGCs). In order to understand the mechanisms involved in this neuroprotective effect, present study aimed to determine the expressions of RNAs and proteins in various treatments. The proteins expressed in the control group, N-methyl-D-aspartate (NMDA)-treated group, and aloe emodin metabolites-cotreated group were separated by two-dimensional gel electrophoresis (2-DE). Protein spots were excised from 2-DE and analyzed by nano-liquid chromatography with mass spectrometry, MS/MS; tandem MS. Quantitative polymerase chain reaction (Q-PCR) was used to investigate the RNA related to these proteins. There were 84 spots with significant differences in various treatments. Among the 84 spots, the authors identified 9 spots whose functions were closely related to regulate the apoptosis of cells. The results of Q-PCR were not completely unanimous with those of 2-DE. The results suggested that aloe emodin metabolites decreased NMDA-induced apoptosis of RGCs by preserving, and inducing, some proteins related to the antioxidation and regulation of cells' energy. Both the level of RNA and protein of superoxide dismutase (Cu-Zn) were significantly elevated after aloe emodin metabolites were added. The mechanisms of neuroprotection are complicated, and involve not only the transcription and stability of mRNA, but also post-translation protein modifications, degradation, and protein-protein interaction<sup>[56]</sup>.

Diabetes mellitus is reported to impair the memory function in experimental animals. Since the mammalian hippocampus and cerebral cortex play a pivotal role in a diverse set of cognitive functions, such as novelty detection and memory, the authors examined the vulnerability of cortex and hippocampus regions of the brain to oxidative damage in streptozotocin (STZ) diabetic mice. The authors examined the attenuating effect of extracts of *Withania somnifera* and *Aloe vera* on prevention of hippocampal and cortical cell degeneration of their total antioxidant activity and also their potency to reduce Fe<sup>3+</sup>.

The authors assayed lipid peroxidation (LPO) and protein carbonyl (PC) in both regions of the brain and observed the changes in memory and motor behavioral functions in diabetic and control mice. The results showed a significant ( $p<0.05$ ) increase in LPO and PC in hippocampus and cortical regions of STZ diabetic mice. The authors also found a significant impairment in both motor and memory behavioral functions in diabetic mice. However, when diabetic mice were supplemented with the extracts of *Withania somnifera* and



*Aloe vera*, the oxidative damage in both brain regions was reduced as marked by significant ( $p < 0.05$ ) declines in both LPO and PC. The combination of extracts of *Withania somnifera* and *Aloe vera* was more effective in reducing oxidative damage in brain regions than the supplementation of single plant extract. The combination also lowered the blood glucose level in comparison to STZ diabetic mice. Memory impairment and motor dysfunction were also improved by the plant extracts supplementation. The authors conclude that impairment in the hippocampus and cortex in STZ diabetic mice are associated with an increased free radical mediated oxidative damage and that the supplementation of plant extracts showed preventive effects in attenuating oxidative damage in both brain regions possibly via antioxidative mechanisms<sup>[57]</sup>.

Anemia is not a normal manifestation of aging. Failure to evaluate anemia in aged population as well as untreated geriatric anemia may result in increased morbidity and mortality including decreased bodily functions. A proper management of anemia has shown to improve several adverse future consequences among the elderly. Iron replacement therapy is restricted due to severe risk profile particularly in reference to aging population. Aged individuals (94 elderly people, males 59 and females 35; aged 62-75 year) showing evidence of iron deficiency anemia completed a three months placebo controlled trial conjugated with an Ayurvedic formulation containing hydro-alcohol extract of *Hippophae rhamnoides* and *Aloe vera* in effective doses and were evaluated on various clinical symptomatology including certain bio-chemical estimations like Hb, RBC, serum protein, serum iron, serum ferritin, inflammatory cytokines (IL-6 and TNF- $\alpha$ ) including c-reactive protein (CRP). The test drug supported in improving clinical complaints and increased the Hb, RBC, serum total protein, iron and ferritin levels. Further, decrease in inflammatory cytokines, IL-6 and TNF- $\alpha$  including CRP following test drug treatment which are known to enhance with advancing age and contribute in the onset of anemia, was in favour of improving the anemia and anemia associated adverse consequences among the aged people. There was no adverse reaction during the trial period<sup>[58]</sup>.

Anti-glycation activity of the extracted from *Aloe sinkatana* and isolated compounds; 2,8-dihydroxy-6-(hydroxymethyl)-1-methoxyanthracene-9,10-dione<sup>[11]</sup>; aloe emodin derivative and known compounds; aloe emodin, feraloide, aloins, etc, was carried out using the hemoglobin- $\sigma$ -gluconolactone and glucose-bovine serum albumin assays. The results obtained showed that methyl alcohol and ethyl acetate extracts as well as compound 1 showed an inhibitory effect on early stage protein glycation. Compound 1 showed significant inhibitory effects against glucose-induced advanced glycation end-products<sup>[59]</sup>.

Anthraquinone derivatives such as emodin have recently been shown to protect in models of  $\beta$ -Amyloid (A $\beta$ ) and tau aggregation-induced cell death. The mechanisms of action possibly involve preconditioning effects, anti-aggregation properties, and/or enhancing the phosphatidylinositol-3-kinase (PI3K)/AKT survival mechanism.

The authors studied several natural (emodin, rhein, and aloin) and synthetic anthraquinone-2-sulfonic acid (AQ<sub>2</sub>S), to screen for post-treatment therapeutic benefit in two models of neuronal death, namely hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and staurosporine (STS)-induced injury. Treatment with emodin, rhein, or aloin failed to reduce H<sub>2</sub>O<sub>2</sub> injury. Moreover, consistent with emodin behaving like a mild toxin, it exacerbated oxidative injury at the highest concentration used (50  $\mu$ M) in the post-treatment paradigm, and potently inhibited AKT. In contrast, AQ<sub>2</sub>S was neuroprotective. It reduced H<sub>2</sub>O<sub>2</sub> injury at 50 and 75  $\mu$ M. In addition, AQ<sub>2</sub>S potently inhibited STS-induced injury. The mechanisms of action involve caspase inhibition and AKT activation.

However, blockade of AKT signaling with LY294002 failed to abolish AQ<sub>2</sub>S-mediated protection on the STS assay. This is the first study to report that AQ<sub>2</sub>S is a new neuroprotective compound and a novel caspase inhibitor<sup>[60]</sup>.

The authors explored the effect of *Aloe vera* in animal models 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 *Aloe vera* significantly reduced the transfer latency as compared to control. The forced swim test as well as tail suspension test showed that *Aloe vera* at all administrated doses (100, 200, and 400 mg/kg, p.o.) decreased 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 *Aloe vera* enhances learning and memory, and also alleviates depression in mice<sup>[61]</sup>. The study on neuroprotective effects of natural polyphenols including anthraquinones and their interaction with specific molecular targets and pathways of neurodegeneration was focused *In vitro* and animal studies.

#### Preliminary clinical study

The authors investigated pilot study that the effect of an aloe polymannose multnutrient complex (APMC) formula on cognitive and immune functioning over 12 months among adults diagnosed with Alzheimer's disease (AD). Subjects participated in an open-label trial; females  $n=28$  and males  $n=6$ , and consumed 4 teaspoons per day of the APMC. The ADAS-cog, MMSE, ADCS-ADL, and SIB were administered at baseline and 3, 6, 9, and 12 months follow up. Cytokines and lymphocyte and monocyte subsets were assessed at baseline and 12 months. The mean ADAS-cog cognition score significantly improved at 9 and 12 months from baseline, and 46% of the sample showed clinically-significant improvement ( $\geq 4$ -point change) from baseline to 12 months. Participants showed significant decreases in tumor necrosis factor- $\alpha$ , vascular endothelial growth factor, and interleukins-2 and -4. CD90+, CD95+CD3+, CD95+ CD34+, CD95+CD90+, CD14+CD34+, CD14+CD90+, and CD14+CD95+ decreased significantly, whereas CD14+ significantly increased. Participants tolerated the APMC supplement with few, temporary adverse reactions. The present results showed improvements in both preliminary clinical and physiological outcomes for a decrease that otherwise has no standard ameliorative remedy<sup>[62]</sup>.

## PREVENTION OF PHOTO-AGING AND POLYCYSTIC OVARIAN SYNDROME (PCOS)

### Prevention of photo-aging

The authors investigated the effect of *Aloe vera* gel and aloe gel constituents on activity of microbial and human metalloproteinases. Clostridium histolyticum collagenase (ChC) results dose-dependently inhibited by aloe gel and the activity-guided fractionation led to an active fraction enriched in phenolics, and aloin  $\alpha$  and  $\beta$ . Aloins have been shown to be able to bind and to inhibit ChC reversibly and non-competitively. Aloe gel and aloins are also effective inhibitors of stimulated granulocyte matrix metalloproteinase (MMPs).

The remarkable structural resemblances between aloins and the pharmacophore structure of inhibitory tetracyclines, suggest that the inhibitory effects of aloins are via an interaction between the carbonyl group at C(9) and an adjacent hydroxyl group of anthracene [C(1) or C(8)] at the secondary binding site of enzyme, destabilizing the structure of granulocyte MMPs<sup>[63]</sup>. The results presented that the increase in the collagen content observed in healing wound with aloe gel is consistent with the ability of aloins and aloe gel components, such as aloesin, glycoprotein and aloe polysaccharides to inhibit collagenase activity.

An examination glove that delivers *Aloe vera* gel to the gloved hand was studied in 30 adult females with bilateral occupational dry skin with or without irritant contact dermatitis (with or without erythema, fissures, and excoriations). **Methods:** All participants were factory assembly-line workers with repeated superficial skin trauma who attributed their dry, irritated, emollient-dependent skin to a common cause (occupational exposure). Participants were sequentially enrolled (after written informed consent,  $n=29$  evaluable participants) into an open, contralateral comparison study to evaluate efficacy of *Aloe vera* glove use 8 h/day to one hand versus no use to the opposite hand for 30 days, followed by 30 days rest, followed by 10 days of repeated use. Participant's dorsal hands were documented by standardized photos at baseline, during, and at the end of study. **Results:** Unblinded investigator baseline assessment rated dry skin as mild to moderate ( $n=27$ ), or moderate to severe ( $n=2$ ). Mean time to noticeable improvement for the *Aloe vera* glove hand was 3.5 days (range: 2-6 days) whereas marked improvement was 10.4 days (range: 7-17 days) for the *Aloe vera* glove hand. No improvement was detected for nonglove hands. Blinded photo assessment was rated independently by dermatology research staff. End-of-study mean global assessment of *Aloe vera* glove hands versus nonglove hands was 1.3 for *Aloe vera* glove hand [0=no change, 1=good (10% -89% global improvement), 2=marked improvement (90% -100% global improvement)] versus 0 for nonglove hand ( $P<0.0001$ ). Mean global end-of-study assessments by the participants=2.0 for *Aloe vera* glove hand versus 0 for nonglove hand. **Conclusion:** Dry-coated *Aloe vera* gloves that provide for gradual delivery of *Aloe vera* gel to skin produced a uniformly positive outcome of improved skin integrity, decreased appearance of fine wrinkling, and decreased erythema in the management of occupational dry skin and irritant contact dermatitis<sup>[64]</sup>.

Ultraviolet (UV) irradiation induces photo-damage of the skin, which in turn causes depletion of the dermal extracellular matrix and chronic alterations in skin structure. Skin wrinkle formations are associated with collagen synthesis and matrix metalloproteinase (MMP) expression. The production of type I procollagen is regulated by transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) expression; the activation of MMP is also correlated with an increase of interleukin-6.

The authors examined whether baby aloe shoot extract (BAE, immature aloe extract), which is from the one-month-old shoots of *Aloe vera*, and adult aloe shoot extract (AE), which is from the four-month-old shoots of *Aloe vera*, have a protective effect on UVB-induced skin photo-aging in normal human dermal fibroblasts (NHDFs). The effects of BAE and AE on UVB-induced photo-aging were tested by measuring the levels of reactive oxygen species, MMP-1, MMP-3, IL-6, type I procollagen, and TGF- $\beta$ 1 after UVB irradiation. It was found that NHDFs cell treated with BAE after UVB-irradiation suppressed MMP-1, MMP-3, and IL-6 levels compared to the AE-treated cells. Furthermore, BAE treatment elevated type I procollagen and TGF- $\beta$ 1 levels. The present results suggest that BAE may potentially protect the skin from UVB-induced

damage more than AE<sup>[65]</sup>.

The authors investigated whether dietary *Aloe vera* gel has anti-aging properties on the skin. **Methods:** Thirty healthy female subjects over the age of 45 were recruited and they received 2 different doses (low-dose: 1,200 mg/d, high-dose: 3,600 mg/d) of *Aloe vera* gel supplementation for 90 days. Their baseline status was used as a control. At baseline and at completion of the study, facial wrinkles were measured using a skin replica, and facial elasticity was measured by an *In vivo* suction skin elasticity meter. Skin samples were taken before and after aloe intake to compare the type I procollagen and matrix metalloproteinase 1 (MMP-1) mRNA levels by performing real-time RT-PCR. **Results:** After aloe gel intake, the facial wrinkles improved significantly ( $p<0.05$ ) in both groups, and facial elasticity improved in the lower-dose group. In the photo-protected skin, the type I procollagen mRNA levels were increased in both groups, albeit without significance; the MMP-1 mRNA levels were significantly decreased in the higher-dose group. Type I procollagen immunostaining was substantially increased throughout the dermis in both groups. **Conclusion:** Aloe gel significantly improve wrinkles and elasticity in photo-aged human skin, with an increase in collagen production in photo-protected skin and a decrease in the collagen-degrading MMP-1 gene expression. However, no dose-response relationship was found between the low-dose and high-dose groups<sup>[66]</sup>.

#### Prevention of polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is recognized as the most common endocrinopathy of women. Increased androgen synthesis, disrupted folliculogenesis, and insulin resistance lie at the pathophysiological core of PCOS. Current therapy for such a syndrome is use of insulin sensitizers. Large randomized clinical trials of metformin as the insulin-sensitizing drug, however, suggested that it produces many side effects after prolonged usage. For this reason, an alternate therapy would be to use herbs with hypoglycemic potential. *Aloe vera* popularly is known as a well-known plant with such properties and the preliminary clinical study of aloe high molecular fractions with metformin showed significant efficacy for type 2 diabetic patients<sup>[3]</sup>.

The authors explored the efficacy of *Aloe vera* gel formulation as a possible therapeutic agent in the prevention and management of PCOS. The present study evaluated the efficacy of *Aloe vera* gel formulation in a PCOS rat model. Five month old Charles Foster female rats were orally fed with letrozole, a non-steroidal aromatase inhibitor, to induce PCOS. The rats were then treated orally with the *Aloe vera* gel formulation (aloin removed *Aloe vera* gel, with curcuma and karaya gum in lemon juice) 1 mL dose daily for 45 days. This restored their estrus cyclicity, glucose sensitivity, and steroidogenic enzymes (3 $\beta$ -HSD and 17 $\beta$ -HSD) activity. Co-treatment of the inductive agent, letrozole, with the *Aloe vera* gel prevented the development of the PCOS phenotype by restoring the ovarian steroid status, and altering key steroidogenic activity. This can be attributed to phyto-components present in the extract<sup>[67]</sup>.

The authors evaluated the lipid correlating effect by *Aloe vera* formulation in a letrozole-induced polycystic ovarian syndrome in rat model. **Material and Methods:** PCOS was induced in Charles Foster female rats by oral administration of non-steroidal aromatase inhibitor letrozole (0.5 mg/kg body weight, 21 days). All rats were hyperglycemic and 90% rats also showed elevated plasma triglycerides, elevated LDL cholesterol levels, and lowered plasma HDL cholesterol levels indicative of a dyslipidemic profile. PCOS positive rats with an aberrant lipid profile were selected for treatment.

An *Aloe vera* gel formulation (*Aloe vera* aloin-removed gel, with curcuma, karaya gum in lemon juice), was orally administered 1 ml, (10 mg/kg), for 21 days. **Results and Conclusion:** *Aloe vera* gel treated PCOS rats exhibited significant reduction in plasma triglyceride and LDL cholesterol levels, with an increase in HDL cholesterol. The gel treatment also caused reversion of abnormal estrous cyclicity, glucose intolerance, and lipid metabolizing enzyme activities, bringing them to normal. In conclusion, *Aloe vera* gel has phytochemicals with anti-hyperlipidemic effects and it has shown efficacy in management of not only PCOS but also the associated metabolic complication: dyslipidemia<sup>[68]</sup>.

## STIMULATION OF PERIODONTAL FIBROBLASTS AND BONE FORMATION, AND HEALING OF PAIN AND NEPHROTOXICITY

### Stimulation of periodontal fibroblasts and bone formation

Periodontal fibroblasts and pulpal fibroblasts have been considered as the major cells responsible for new alveolar bone and dentin formation, respectively. Bone morphogenetic protein-2 (BMP-2) has great clinical potential for inducing bone and dentin repair and regeneration.

The authors investigated the effect of acemannan, polysaccharide fraction of *Aloe vera* gel on BMP-2 expression in primary human periodontal fibroblasts and pulpal fibroblasts. Cells were treated with increasing concentration of acemannan for 24 and 48 hours. The reverse transcriptase-PCR (RT-PCR) and ELISA assays were used to investigate the effects of acemannan on the levels of BMP-2 mRNA and protein expression, respectively. The results from RT-PCR and ELISA revealed that acemannan induced BMP-2 mRNA level and protein synthesis in both cells. The effective concentrations of acemannan were 0.25-1 mg/mL. Collectively, these results suggest that acemannan is a potent plant polysaccharide for stimulating BMP-2 expression in periodontal fibroblasts and pulpal fibroblasts<sup>[69]</sup>.

The authors investigated the effect of acemannan on dentin formation. Primary human dental pulp cells were treated with acemannan. New DNA synthesis, bone morphogenetic protein-2 alkaline phosphatase activity, dentin sialoprotein expression, and mineralization were determined by <sup>3</sup>H-thymidine incorporation, enzyme-linked immunosorbent assay, biochemical assay, western blotting, and alizarin red staining, respectively. Then, the upper first molars of 24 male Sprague Dawley rats were intentionally exposed and capped with either acemannan or calcium hydroxide. At day 28, the teeth were histopathologically examined and evaluated for the degree of inflammation, dentin bridge formation, and pulp tissue organization. The results revealed that acemannan significantly increased pulp cell proliferation, bone morphogenetic protein-2, alkaline phosphatase activity, dentin sialoprotein expression, and mineralization, compared with the untreated group. The acemannan-treated group also exhibited a complete homogeneous calcified dentin bridge and good pulp tissue organization, whereas neither was detected in the calcium hydroxide-treated and sham group. In the acemannan-treated group, either mild or no inflammation was found, whereas the other groups had various degrees of inflammation. The data suggest that acemannan promotes dentin formation by stimulating primary human dental pulp cell proliferation, differentiation, extracellular matrix formation, and mineralization. Acemannan also has pulpal biocompatibility and promotes soft tissue organization<sup>[70]</sup>.

The authors investigated bone formation of acemannan in a tooth

extraction rat model.

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 thymine 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>[71]</sup>.

The authors investigated effect of acemannan as a bioactive molecule and scaffold for periodontal tissue regeneration. Primary human periodontal ligament cells were treated with acemannan *In vitro*. New DNA synthesis, expression of growth/differentiation factor 5 and runt-related transcription factor 2, expression of vascular endothelial growth factor, bone morphogenetic protein-2 and type I collagen, alkaline phosphatase activity, and mineralized nodule formation were determined using <sup>3</sup>H-thymidine incorporation, reverse transcription-polymerase chain reaction, enzyme-linked immunosorbent assay, biochemical assay and alizarin red staining, respectively. *In vivo* study, premolar class II furcation defects were made in four mongrel dogs. Acemannan sponges were applied into the defects. Untreated defects were used as a negative control group. The amount of new bone, cementum and periodontal ligament formation were evaluated 30 and 60 days after the operation. **Results:** Acemannan significantly increased periodontal ligament cell proliferation, upregulation of growth/differentiation factor 5, runt-related transcription factor 2, vascular endothelial growth factor, bone morphogenetic protein 2, type I collagen and alkaline phosphatase activity, and mineral deposition as compared with the untreated control group *In vitro*. Moreover, acemannan significantly accelerated new alveolar bone, cementum and periodontal ligament formation in class II furcation defects. The present data suggest that acemannan could be a candidate biomolecule for periodontal tissue regeneration<sup>[72]</sup>.

The tendon is composed of highly organized collagen fibers that form a complex supramolecular structure. After lesion, the organization and composition of the tendon are not completely restored. The authors evaluated if the application of *Aloe vera* improves tendon healing, considering the effectiveness in the stimulus of collagen synthesis. **Main methods:** The calcaneal tendon of male Wistar rats was partially transected with subsequent topical application of *Aloe vera* ointment at the injury. The animals were separated into groups with tendons treated with the *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 *Aloe vera* extract. **Key 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 high birefringence in tendons of the plant-treated group on 21<sup>st</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 after lesion and the active isoform of MMP-2 increased on day 21 in plant-treated groups. 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>[73]</sup>.

### Healing of pain and nephrotoxicity

The authors explored the effect of aqueous extract of *Aloe vera* gel on behavioural parameters of pain. Pain assessment was performed by the tail-flick and formalin tests. *Aloe vera* gel extract containing 3.14% aloin was dissolved in distilled water to prepare suspensions of required dose of 100 mg, 200 mg and 400 mg/kg. *Aloe vera* (100 mg/kg, per oral) produced an insignificant decrease in the pain response in the tail-flick and formalin tests. Moreover, *Aloe vera* (200 mg and 400 mg/kg, p.o.) did not have significant effect on the tail-flick test. However, *Aloe vera* (200 mg and 400 mg/kg, p.o.) significantly decreased the second phase of the formalin-induced pain. Thus, these findings suggest that *Aloe vera* exerts its effect by a peripheral mechanism of action rather than central<sup>[74]</sup>.

The study was carried out using male Wistar rats (150-200 g). The animals were divided into 5 groups ( $n=6$ ) receiving different treatments. Both visceral and somatic pain in animals was assayed using radiant heat method, hot plate method and writhing test. The first group of animals was taken as control, the second group was given the reference standard drugs and the other groups received indigenous drug *Aloe vera* gel at different doses. For sub-acute toxicity study, *Aloe vera* was administered daily for 14 days at the dose level of 300 mg/kg dose. Biochemical analysis of blood and histo- pathological study of gastrointestinal (GI) mucosa was done after 14 days. Aqueous extract of *Aloe vera* gel showed significant analgesia compared to control. The results were significant ( $p<0.001$ ) in radiant heat method (tail flick type) and also in hot plate method ( $p<0.05$ ) at the dose of 300 mg/kg. Writhing test (induced with 4% NaCl, i.p. injection) showed maximum inhibition (51.17%) at the dose of 300 mg/kg. No adverse effects on renal and hepatic functions were found with *Aloe vera*. Histo-pathological study of GI mucosa showed preservation of normal architecture with *Aloe vera*. The aqueous extract of *Aloe vera* gel showed significant analgesia and to be safe in respect of the renal and hepatic functions along with no adverse effects on GI mucosa. Gastroprotective effects of *Aloe vera* gel may be used as an analgesic<sup>[75]</sup>.

The authors analyzed the effect of *Aloe vera* nephrotoxicity induced by soybean oil in rats kidney. **Material and methods:** Eighteen Wistar Albino female rats were used for the experiment in 3 groups (control group, experimental group and vehicle group). *Aloe vera* at a dose of 140 mg/kg/day was dissolved in soybean oil and administered as 500 mg capsule form. Soybean oil (500 mg/kg) was administered to the vehicle group. All administrations were given orally by gavage between 10-11 am for three weeks. Biopsy materials were taken the right kidneys of the rats and were analyzed under the light microscope. **Results:** Biopsy materials, which were taken from the kidneys of the control subjects, revealed normal structural features of renal cortex and medulla. No distinctive difference was found in the Malpighi corpuscles in the group receiving *Aloe vera*. However, various degrees of vacuolization in the proximal tubules, and the complete loss of structural characteristics of proximal tubules

were observed. Vasculization was rarely seen in distal tubules. Congestion was clear in medulla. In the third group, receiving only soybean oil, a distinctive congestion was observed in all glomerulus and in the medulla. The detected pathologic changes were more common and severe in the third experiment group that received only soybean oil. Congestion in glomerulus was mostly observed in this group. **Conclusion:** These results show the conclusion that *Aloe vera* plays a healing role against the toxic effects of soybean oil on the kidney<sup>[76]</sup>.

Significant degenerative changes were observed in the kidney tissue of untreated neonatal streptozotocin-induced type 2 diabetic rats. These degenerative changes were diminished in the kidney tissue of diabetic animals given glibenclamide and *Aloe vera* gel and pulp extracts. Kidney lipid peroxidation levels were increased in diabetic rats compared to healthy rats; these levels were higher in rats treated with glibenclamide than in those which received aloe extracts. Serum urea and creatinine levels were higher in diabetic rats in comparison to healthy rats. The administration of aloe gel extract and glibenclamide decreased serum urea and creatinin levels in comparison to diabetic controls. Only *Aloe vera* leaf gel extract showed improvement both in histological and biochemical parameters suggesting a protective effect of *Aloe vera* on mild damage caused by type 2 diabetes on kidney tissue<sup>[77]</sup>.

The authors evaluated the ability of aqueous extract of *Aloe vera* on oxidative damage and anion exchanger 1 (AE1, also known as Band 3) expression in human erythrocytes exposed to the water soluble free radical initiator 2,2'-azobios-2-amidinopropano dihydrochloride (AAPH). In addition, total phenolic compounds in the extracts were determined as catechin equivalent and the various antioxidant activities were compared to natural and synthetic standard antioxidants such as BHA and ascorbic acid. Since *Aloe vera* extract did not cause a consumption of the cytosolic antioxidant, glutathion (GSH) when it was direct incubated with GSH in basic aerated aqueous solution, this indicates that *Aloe vera* extract does not proceed auto-oxidation at this experimental condition. Furthermore, *Aloe vera* extract prevent the consumption of GSH, in radical treated RBCs. It also inhibits consumption of GSH when it was direct incubated with AAPH. *Aloe vera* gel extract inhibits the the generation of diphenyl-2-picrylhydrazyl (DPPH) and the scavenging activity was increased in a dose dependent manner. *Aloe vera* extract was shown the similar reducing power than standards BHT and ascorbic acid. Biochemical analysis by SDS-PAGE and western blotting showed that AAPH-induced oxidative stress increased the susceptibility of AE1 to proteolytic degradation. Of note, the data evidenced that *Aloe vera* treatment was able to partially restore the normal RBC membrane protein profiles in a dose-dependent manner. These results clearly demonstrate the antioxidative activity of *Aloe vera* gel extract that might be ascribed to be a synergistic action of the bioactive compounds contained therein<sup>[78]</sup>.

The author investigated to identify, quantify, and compare the phytochemical contents, antioxidant capacity, and antibacterial activities of *Aloe vera* lyophilized leaf gel (LGE) and 0.5% ethanol leaf gel extract (ELGE) using GC-MS and spectro-photometric methods. **Results:** Analytically, 95% ethanol is less effective than ethyl acetate/diethyl ether or hexane (in case of fatty acids) extractions in separating phytochemicals for characterization purposes. However, although fewer compounds are extracted in the ELGE, they are approximately 345 times more concentrated as compared to the LGE, hence justifying ELGE use in biological efficacy studies *In vivo*. Individual phytochemicals identified included various phenolic acids/polyphenols, phytosterols, fatty acids,

indoles, alkanes, pyrimidines, alkaloids, organic acids, aldehydes, dicarboxylic acids, ketones and alcohols. Due to the presence of the antioxidant polyphenols, indoles and alkaloids, the *Aloe vera* leaf gel shows antioxidant capacity as confirmed by oxygen radical absorbance capacity and ferric reducing antioxidant power analyses. Both analytical methods used show the non-flavonol polyphenols to contribute to the majority of the total polyphenol content. Three different solvents such as aqueous, ethanol, and acetone were used to extract the bioactive compounds from the leaves of *Aloe vera* to screen the antibacterial activity selected human clinical pathogens by agar diffusion method. The maximum antibacterial activities were observed in acetone extracts other than aqueous and ethanol extracts. **Conclusion:** Due to its phytochemical composition, *Aloe vera* leaf gel may show promise in alleviating symptoms associated with/or prevention of cardiovascular diseases, cancer, neurodegeneration, and diabetes<sup>[79]</sup>.

Among the phytochemicals in *Aloe vera*, aloin is the main constituent and is estimated at levels from 0.1 to 0.66% of leaf dry present in cells adjacent to the rind of the leaf in gel. Hydrophilic aloin, which is metabolized into hydrophobic aloe emodin in human intestinal gut<sup>[8]</sup>, may have putative prophylactic efficacies as followed; anticancer, anti-inflammatory, antioxidant and antimicrobial activities, besides laxative drug, and is expected to be a candidate for a putative prodrug<sup>[80]</sup>.

In combination of phytochemicals with various medicinal properties; phenolics, vitamins, minerals, and acemannan with the immunomodulatory activity through activation of immune responses, *Aloe vera* gel may strongly express some synergistic properties with its medicinal uses<sup>[81]</sup>.

## ANTI-INFLAMMATION AND IMMUNO-RESPONSE

Due to the chronic nature of disease, diabetes, cardiovascular and atherosclerosis have to maintain a healthy lifestyle that includes lifestyle modification and nutrition diet, supporting the immune system to protect the body against infection. Possible efficacies of acemannan and *Aloe vera* high molecular weight fractions as carbohydrate-based adjuvants to immune responses were reported in a previous review<sup>[7]</sup>.

The authors reported the effect of *Aloe arborescens* var. *natalensis* (aloe extract) on peripheral phagocytosis in adult bronchial asthma as following: **Sample preparation:** Five ml of a 20% solution of aloe extract in saline was prepared and administered orally twice a day for 24 weeks. **Response to aloe extract by chronic asthmatic patients:** Clinical assessments were obtained at the beginning and end of the 4-w basal period and 2-w intervals thereafter. At the conclusion of the 24-w period, the patient was given an option to continue or discontinue treatment by means of the following questionnaire: Would you prefer to: (1) stop treatment? (2) continue with the extract from the first 12-w period? (3) continue with the extract from the second 12-w period? (4) no special preference. Physician evaluation forms were completed at the end of each 2-w period and were completed at the end of the 24-w treatment with reference to the patients' symptom diary readily given at the beginning of this trial. Physician evaluation was based on the following scheme: (1) no change; (2) slightly better; (3) very much better; (4) slightly worse; (5) very much worse. In order to fairly evaluate the extract, only responses to questions No.3 were considered positive. There was no adverse effect through the 24-w trial period. **Results:** Oral administration for six months of aloe extract showed efficacy for chronic bronchial asthma of various ages

as well as intrinsic types. Aloe extract was effective in 33.3% cases in chronic asthma patients, but an important finding was that aloe extract was not efficacious at all for the patients who had previously been administered corticosteroid. These findings suggest that aloe extract may be involved in a restoration of protective mechanisms followed by augmentation of immuno-potentiality in adult bronchial asthma<sup>[82]</sup>.

Applications of natural polymeric adjuvants such as acemannan and lentinan have the ability not only to activate humoral but also cellular immune responses in the host. The depot effect, which involves slow release of antigen over a long duration of time, using different forms of polymers, and enhances the co-stimulatory signals for optimal immune activation is the underlying principle of their adjuvant properties.

The authors suggested that polymeric adjuvants may interact and activate various toll-like receptors and inflammasomes, thus involving several innate immune players in the ensuing immune responses<sup>[83]</sup>.

### Protective effect of *Aloe vera* on polymicrobial sepsis in mice

Sepsis is an acute life-threatening clinical condition and remains the major cause of death in intensive care units. The primary pathophysiological event central to the septic responses is an overwhelming activation of the inflammatory system and countervailing response from the anti-inflammatory system.

The authors reported that *Aloe vera* therapeutically reverses the lethality induced by cecal ligation and puncture, a clinically relevant model of sepsis. The administration of *Aloe vera* ameliorated the multiple organ dysfunction syndrome, as evidenced by the serum levels of biochemical parameters and histological changes. In order to investigate the pharmacological mechanism of *Aloe vera*, the levels of the cytokines, tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , and IL-6 were determined by ELISA at various time points. The increases in the levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were attenuated by *Aloe vera*. *In vivo* administration of *Aloe vera* also markedly enhanced bacterial clearance. It was suggested that *Aloe vera* could be a potential therapeutic agent for the clinical treatment of sepsis<sup>[84]</sup>.

### The effect of *Aloe vera* leaf gel in hypercholesterolemic and anti-lipidemic animal, and hyperlipidemic type 2 diabetic patients

Atherosclerosis is a complex disease that is associated with a variety of etiologic factors such as hyper-lipidemia and inflammation. The beneficial effects of *Aloe vera* leaf gel on some of the atherosclerosis risk factors, and also fatty streak formation in hypercholesterolemic rabbits were defined. **Materials and Methods:** 32 white male rabbits were randomly divided into four experimental groups ( $n=8$ , each). During the study, the animals had a standard diet (control group), high cholesterol diet (HC group), high cholesterol diet with *Aloe vera* leaf gel (3.2%v/v) (HC+aloe group) and *Aloe vera* leaf gel (aloe group) for 30 days. Fasting blood samples were collected from all animals at the beginning and end of the study. Then total cholesterol (TC), fasting blood sugar (FBS), triglyceride (TG) and C-reactive protein (CRP) were measured before and after experimental periods. By the end of the study, the aortas were removed and investigated for atherosclerosis plaque formation. **Results:** Significant differences were observed in TG and CRP levels of the high-cholesterol diet with *Aloe vera* and the high-cholesterol diet alone ( $p<0.05$ ). The formation of fatty streaks in the aorta was also significantly lower in the same animal under the influence of dietary *Aloe vera* ( $p<0.05$ ). The control and *Aloe vera* group did not show any evidence of atherosclerosis. No significant difference was found between the groups in TG and

**FBS. Conclusions:** The data suggest that *Aloe vera* has beneficial effects on the prevention of fatty streak development; it may reduce the development of atherosclerosis through modification of risk factors<sup>[85]</sup>.

The total phenolic and flavonoid contents, the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging ability and the ferric reducing power (FRAP) of *Aloe vera* were measured to determine the antioxidant activity of this species. The *In vivo* antidiabetic effects of *Aloe vera* were also investigated using streptozotocin-induced type 2 diabetic model rats that were divided into five groups based on the treatment received: (1) water (WC); (2) glibenclamide; (3) concentrated gel extract (Gel-C); (4) ethanol (80%) gel extract (Gel-Et); and (5) ethanol (80%) skin extract of *Aloe vera* (Skin-Et). Skin-Et, which contained the highest level of total phenolics (62.37±1.34 mg/kg) and flavonoids (20.83±0.77 mg/kg), exhibited the highest scavenging activity (85.01±0.52 %) and the greatest reducing power (185.98±0.41 µM), indicating that the skin contained the highest level of antioxidants. The oral consumption of Gel-Et for 4-w caused significant reduction in the fasting serum glucose levels of the rats. The rats in the Gel-C-, Gel-Et- and Skin-Et-treated groups experienced a reduction in their total cholesterol levels by 11%, 17%, and 25%, respectively and a reduction in their LDL cholesterol levels by 45%, 3%, and 69%, respectively. The *In vivo* experimental antioxidant parameter MDA is strongly correlated with the *In vitro* antioxidant parameters of flavonoids and polyphenols, namely the DPPH and FRAP values ( $r=0.94, 0.92, 0.93, 0.90$ ), thus confirming the antioxidant potential of the *Aloe vera* extracts<sup>[86]</sup>. The association of hyperglycemia with an alteration of lipid parameters presents a major risk for cardiovascular complications in diabetes. Hence, an improvement in the lipid profiles will reduce diabetic complications.

The randomized double-blind placebo-controlled clinical trial with hyperlipidemic (hypercholesterolemic and/or hypertriglyceridemic) type 2 diabetic patients aged 40-60 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 vera* 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 ( $p=0.036, p=0.036, p=0.006, \text{ and } p=0.004$ , 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>[87]</sup>.

The authors demonstrated that aloe emodin dose-dependently inhibited inducible nitric oxide synthase (iNOS) mRNA expression and nitric oxide (NO) production at 5-40 µM. In addition, the levels of cyclooxygenase-2 (COX-2) mRNA and prostaglandin E2 (PGE2) production were suppressed by 40 µM aloe emodin. Aloin also suppressed the production of NO at 5-40 µM, although it did not suppress PGE2 production. The present results indicate that aloin and aloe emodin possibly suppress the inflammatory responses by blocking iNOS and COX-2 mRNA expression. The anti-inflammatory effect of aloe emodin was comparable to that of kaempferol and quercetin, indicating aloe emodin as a possible key constituent responsible for the anti-inflammatory activity of aloe<sup>[88]</sup>.

#### ***Aloe vera* downregulates LPS-induced inflammatory cytokine**

#### **production and expression of NLRP3 inflammasome in human macrophages**

*Aloe vera* has been used in traditional herbal medicine as an immunomodulatory agent inducing anti-inflammatory effects. IL-1β production is strictly regulated both at transcriptional and posttranslational levels through the activity of Nlrp3 (Nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3) inflammasome.

The authors showed for the first time that *Aloe vera*-mediated strong reduction of IL-1β appears to be the consequence of the reduced expression of both pro-IL-1β as well as Nlrp3 inflammasome components via suppressing specific signal transduction pathways. The authors found that the expression of the ATP sensor purinergic receptor P2X ligand-gated ion channel 7 (P2X7) receptor is also downregulated by *Aloe vera* that could contribute to the attenuated IL-1β cytokine secretion. Interfering with the cytokin overproduction during early sepsis or in chronic inflammatory or autoimmune disease may improve the outcome and quality of life of patients. These results may provide a new therapeutic approach to regulate inflammasome-mediated responses. *Aloe vera* could be a new therapeutic tool to target Nlrp3 inflammasome-mediated cytokine production<sup>[89]</sup>.

Recent study suggests that Nlrp3 inflammasome activation is elevated in myeloid cells from type 2 diabetic patients (drug-naive patients:  $n=47$ , control:  $n=57$ ) and that antidiabetic treatment with the anti-diabetic drug, metformin, contributes to modulation of inflammasome activation in type 2 diabetes<sup>[90]</sup>.

In an earlier preliminary clinical study, *Aloe vera* gel with hypoglycemic medication, metformin, significantly decreased in blood glucose- and triglyceride-level, and HbA1C value on type 2 diabetic patients. *Aloe vera* gel high molecular fractions, containing less than 10 ppm of aloin and acemannan with glycoprotein, verectin, may possibly contribute to modulation of type 2 diabetic treatment with metformin<sup>[3]</sup>.

#### ***Aloe vera* extract activity on human corneal cells**

Ocular diseases are currently an important problem in modern societies. Patients suffer from various ophthalmologic ailments namely, conjunctivitis, dry eye, dacryocystitis or degenerative diseases. Therefore, there is a need to introduce new treatment methods, including medicinal plants usage.

The present study analyzes the effect of *Aloe vera* extracts obtained with different solvents on *In vitro* culture of human 10.014 pRSV-T corneal cells. **Materials and methods:** NR (neutral red) uptake, MTT, DPPH reduction, Griess reaction, ELISA and rhodamine-phalloidin staining were used to test toxicity, anti-proliferative activity, reactive oxygen species (ROS) reduction, nitric oxide (NO) and cytokine level, and distribution of F-actin in cells, respectively. **Results:** It was found that the extract of *Aloe vera* obtained with different solvents on *In vitro* culture of human corneal cells. No ROS reducing activity by heptane extract and trace action by ethanol extract of *Aloe vera* was observed. Only ethyl acetate extract expressed distinct free radical scavenging effect. Plant extract decreased NO production by human corneal cells as compared to untreated controls. The cytokine (IL-1β, IL-6, TNF-α and IL-10) production decreased after the addition of *Aloe vera* extract to the culture media. **Discussion and conclusion:** *Aloe vera* contains multiple pharmacologically active substances which are capable of modulating cellular phenotypes and functions. *Aloe vera* ethanol and ethyl acetate extracts may be used in eye drops to treat inflammations and other ailments of external parts of the eye such as the cornea<sup>[91]</sup>.

The study of *Aloe vera* on IL-1β inflammatory cytokine production

may provide a new therapeutic approach in lifestyle diseases, such as type 2 diabetes, atherosclerosis and gout, to regulate inflammasome-mediated responses.

### Immune response

Oral administration of *Aloe vera* juice and  $\beta$ -glucan after vaccination in dogs stimulated both cellular (CD 3, CD 4, CD 8 T lymphocyte and B lymphocyte) and humoral (Ig G, Ig M) immune responses, and increased in platelet counts, suggesting the restorative effects on thrombocytopenia<sup>[92]</sup>.

Oral administration of *Aloe vera* polysaccharides positively affected various aspects of the immune system, including the effects on the composition of lymphocyte subsets (CD<sup>4+</sup>, CD<sup>8+</sup>) and serum immunoglobulins (Ig M, Ig G) in rabbit. These findings demonstrate that *Aloe vera* polysaccharide may stimulate both cellular and humoral immune responses after immunization<sup>[93]</sup>. The observed changes after administration of aloe polysaccharide following immunization might occur with similar mechanisms.

The authors explored the effect of aqueous extract of *Aloe vera* on parameters of humoral and cell-mediated immunity in rats.

**Materials and Methods:** Delayed-type hypersensitivity 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* (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>[94]</sup>. The investigations might be useful to clarify the stimulation mechanisms through investigation of Th1 and Th2 helper cells ratios, cellular and humoral immune system for specific cytokines.

## CONCLUSION

The influence of long-term *Aloe vera* ingestion on age-related disease in male Fischer 344 rats was established as follows: (1) long-term *Aloe vera* ingestion lightens the disease burden during the aging process; (2) *Aloe vera* injection slightly suppresses the occurrence of fatal chronic nephropathy, and thrombosis in the atrium of the heart; and (3) no harmful effects or changes in physiological parameters by life-long *Aloe vera* ingestion were observed<sup>[2]</sup>.

A life-long intake of *Aloe vera* gel supplementation in aged rats had superior anti-oxidation action against lipid peroxidation *In vivo*, as indicated by reduced levels of hepatic phosphatidyl choline hydroperoxide. Additional anti-oxidative action was evidenced by enhanced super oxide dismutase and catalase activity. Furthermore, hepatic cholesterol significantly increased in the control group during aging in contrast to the aloe supplemented groups, which showed approximately 30% lower cholesterol levels, thereby an effective hypocholesteremic efficacy. A life-long dietary *Aloe vera* supplementation suppressed free-radical induced oxidative damage and age-related increases in hepatic cholesterol<sup>[95]</sup>.

Recent animal study on supplementation of *Aloe vera* gel with microbiota presented the antihypercholesterolemic effect of the probiotic *Lactobacillus rhamnosus* GG (LGG) plus *Aloe vera* gel in rats with induced hypercholesterolemia, where serum cholesterol, LDL, VLDL, and TAG levels were found to be decreased significantly, and HDL cholesterol levels were observed

to be increased significantly. An optimized blend of the probiotic LGG and *Aloe vera* gel could be exploited as a potential herbo-probiotic therapy to decrease cholesterol levels and lower the risk of cardiovascular disease, although the field is open for further studies<sup>[96]</sup>.

Natural health product (NHP) or comprehensive and alternative medicine (CAM) use is common in patients admitted with acute cardiac-vascular disease. However, health professionals, such as pharmacist, physician and nurse do not commonly identify NHP or CAM as part of the medication profile despite its potential importance<sup>[97]</sup>. Better education of patients and communication between patients and medical practitioners are needed to improve understanding of the risks and benefits of NPH or CAM use.

Aging process and degenerative diseases associated with it are attributed basically to the deleterious side attacks of free radicals on cell constituents and on the connective tissues, damaging consequence of free radical action; lipid peroxidation, DNA damage and protein oxidation. That the process of aging may result, at least in part, from radical-mediated oxidative damage was proposed more than 50 years ago<sup>[98]</sup> and there is growing evidence that aging involves, in addition, progressive changes in free radical-mediated regulatory processes that result in altered gene expression<sup>[99]</sup>.

The prevention for age-related diseases may be effected through multiple mechanisms involving efficient lipid utilization, prevention of neurodegeneration, regeneration of neurofibers, and upregulation of antioxidation enzymes. There is no doubt that antioxidants are necessary components for our health benefits, and the antioxidants and free radical production should be in balance and homeostasis.

It may safely be assumed that antioxidative phytochemicals and acemannan in *Aloe vera* could cooperatively contribute, at least in part, to the improvement of health by preventing of age-related diseases and slowing aging processes through their synergistic systems.

Future investigations on the exploration of health maintenance with *Aloe vera* in long-term ingestion influenced by the intestinal microbiota during aging<sup>[100]</sup>, the outstanding tool and evaluation methods to measure gut flora providing a comprehensive view of the microbial community and its metabolic efficiencies<sup>[101]</sup>, and the modulation of anti-inflammatory processes at the cellular, humoral and molecular levels are extensively required to verify prophylactic efficacy of *Aloe vera* long-term ingestion for age-related diseases.

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## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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