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Aqueous Extract of Human Placenta as a Therapeutic Agent

Piyali Datta Chakraborty¹ and Debasish Bhattacharyya^{2,*}

¹*Albert David Ltd, Kolkata,*

²*Division of Structural Biology and Bioinformatics,
CSIR- Indian Institute of Chemical Biology, Kolkata,
India*

1. Introduction

Traditional folk medicine makes good use of the flora and fauna of a region, including a number of mineral substances. These herbs and animals are used either whole or in part and made up in either simple or complicated preparations and have proved to be effective therapeutic remedies. Some of these medicinal substances of animal origin have been quite thoroughly studied with modern scientific methods, and their therapeutic properties have been confirmed although not always explained. Many others have yet to be subjected to scientific analysis and tests before their real value can be judged. Products of animal origin despite of their therapeutic values have been banned in many countries for the sake of sustainable development in addition to safeguard ecological balance.

It is known from traditional folk knowledge that the placenta, supporting the baby's growth and development in the mother's womb, contains a wide range of biologically active components. Research over decades has been uncovering more and more of these compounds. Indeed, it is claimed that the placenta is capable of producing just about any substance found in any organ of the body. This biochemical treasure house supplies the growing fetus with substances that the fetus itself cannot synthesize. Though a rich source of bioactive components unless recovered, placenta becomes a biomedical waste immediately after childbirth. Use of human placenta as a therapeutic agent, therefore, in no way hampers ecological balance rather promotes resource recovery from a designated biomedical waste. Since most of the natural products of medicinal value have a vast repertoire of potent biological components, there has been an increasing realization to shun synthetic and semi-synthetic medicines primarily because of their harmful side effects.

Research on human placental extract gained a momentum with the description of the preparation of its extract by Russian ophthalmologist Prof. V.P. Filatov. Prior to his research, there were no documents of therapeutic efficacy of the extract, though its use was popular in Europe and parts of Asia primarily China, Korea and Japan for over a century. Review of placenta therapy reveals its long usage from the days of distant past. Recent medical history

* Corresponding author

from 1912 showed that Filatov started research on grafting human corneas using the principle of transplantation of preserved material. He observed that when animal or vegetable tissues are isolated from the organism and subjected to the action of environmental factors that inhibit their vital processes, they undergo a biochemical readjustment. In consequence to this readjustment, the tissues develop substances that stimulate their vital processes. Filatov named these substances as *Biogenic stimulators* (Filatov, 1951). After many clinical experiments, Filatov was convinced that any tissue of human or animal origin could be used to obtain curative effect and this tissue may not necessarily correspond histologically to the tissue affected by the pathological process. He extended this principle to general medicine and confirmed that the process was just as valid for other human tissues. That is how the principle of therapeutic tissue was born (Filatov, 1955).

Placenta serves as a natural storehouse of many biologically active components with significant healing attributes (Tonello et al., 1996). Various extracts of placenta have been described, however, only an aqueous extract of fresh full term human placenta acts as a potent biogenous stimulator (Wu et al., 2003). Over a period of time, it has been demonstrated that only an aqueous extract of human placenta has effective therapeutic potential. The composition of the extracts depends on the method of its preparation and consequently, they show different therapeutic activities. Clinical efficacy of an aqueous extract of human placenta in wound healing is already established (Hong et al, 2010, Wu et al., 2003). Globally scientists are investigating on placenta as a total organ and they expect to discover new applications exploiting the potency of the organ and identify new compounds in near future. This aqueous extract finds importance owing to its ability in curing chronic non-healing wounds including post-surgical dressings and high degree of burn injuries (Wu et al., 2003).

Wound healing is a complicated interrelated process wherein the injured dermal and epidermal tissue is naturally regenerated. Though well regulated, the process of healing is susceptible to interruption or failure leading to the formation of chronic non-healing wounds. Factors which may contribute to this include diabetes, venous or arterial disease, old age and infection. Placental extract, ever since its usage has been shown to be clinically effective in healing normal as well as infected wounds (Shukla et al., 2004, Chakraborty et al., 2009). It is involved in almost every stage of healing. It is also used in wound dressings to speed up the process of recovery. Though clinically well-tested, emphasis is now being laid in understanding the bioactive components involved in the process of healing. Research on the extract has highlighted some important components that might play roles in this process while few more are yet to be identified. Once the active components are identified and a proper biochemical basis of their action is defined, these components may be used even for newer purposes (Chakraborty et al., 2009).

The way in which biological products are produced, controlled and administered requires necessary precautions. Unlike conventional pharmaceutical products, which are normally produced and controlled using reproducible chemical and physical techniques, biological products are manufactured by methods involving biological processes and materials, such as cultivation of cells or extraction of material from living organisms. These processes display inherent variability, so that the range and nature of by-products are also variable. This variability, commercially termed as batch variation, occurs to a higher degree in

products of plant origin compared to those of animal origin. Thus in the manufacture of biological products for direct application to human, full adherence to GMP (Good Medical Practice) is necessary for all production steps, beginning with the starting material from which the active ingredients are produced (WHO, 1992).

For any drug to make its mark in the international market, certification from regulatory authority to ensure its safe use without side effects is compulsory. Exhaustive research to verify the pharmacological efficacy of the drugs derived from traditional medicine in pharmaceutical and biomedical arena is necessary. This would enable to confirm with ancient texts and to understand and verify how the specific pharmacological action of the drug is manifested. Research on the extract reveals ample opportunity for discovery of potent bioactive components whose precise mechanism of action is yet to be elucidated. In the present era of modern pharmaceutical and biomedical research, it has become necessary to verify the pharmacological effects of the drugs in order to check whether they correspond to the ancient texts and to study how the drugs work, and also to isolate the active principles that result in the specific pharmacological action of the extract. In this review, we aim to discuss the efficacy and the biochemical components present in the aqueous extract of human placenta, which has potent wound healing attributes.

2. Preparations of human placental extract

Placental extracts can be classified into two different types: aqueous extract and hydroalcoholic extract. The components present in the extract depend on the method of its preparation and are based on solubility of the components in respective solvent of extraction. Thus, an aqueous extract is likely to contain more polar molecules such as peptides/proteins, small organic components like amino acids, nucleotides, polydeoxyribonucleotides (PDRNs), carbohydrates and trace amount of lipids mostly bound to proteins which are comparatively soluble in aqueous medium. Likewise, various types of lipids may be present in hydroalcoholic extract (less polar and hydrophobic). Chemical analysis of the hydroalcoholic extract revealed the presence of glycosphingolipids, cholesterol, triglycerides, high density lipoproteins, carbohydrates, sialic acids and others, including amino acids, nucleotides, carotenes, vitamins, including small amount of low-molecular-weight proteins/peptides containing hydrophobic amino acid residues which are soluble in a less polar solvent.

Modern indigenous aqueous placental extract is prepared employing Filatov's procedure. The manufacturing procedure of the indigenous extract holding confidentiality of the proprietary terms is as follows: fresh placentae were stored in ice and portions were tested for HIV antibody and Hepatitis B surface antigen. Single hot and cold aqueous extractions were done after incubating dissected and minced placenta at 90°C and 6°C respectively. This was followed by sterilization of the extract under saturated steam (pressure 15-lbs/sq inch at 120°C for 40 min). After filtration and addition of 1.5% (v/v) benzyl alcohol as preservative, ampoules were filled and sterilized once again under the said condition for 20 min. In the first sterilization, the extended duration of heat treatment essentially completed precipitation of a number of macromolecules like proteins. This is apart from adding safety margins to the temperature, time or both to destroy most resistant spore-producing species like *Clostridium tetani*. The terminal sterilization step was to maintain sterility of the products after they were filled and sealed in ampoules. Each milliliter of the drug was

derived from 0.1 g of fresh placenta. A single batch was prepared from the pool of several placentae. The trade name of the extract is 'Placentrex'. Carried over bioactive components in the extract depends on the method of its preparation. As the extract is prepared by repeated sterilization process, it is expected that it may contain macromolecules in their degraded forms together with small bio-organic compounds such as amino acids, peptides, small sized polypeptides, nucleotides, small polynucleotide fragments etc. Only those molecules that are heat stable and are able to withstand high sterilization pressure will remain biologically active. The active components with significant biological activity have been discussed later on. Presence of various organic components gives the extract a yellowish tinge (Datta and Bhattacharyya, 2004a).

In India, several studies have been made on the drug used as wound healer. The extract is a potent biogenous stimulator and abundantly used as an efficient wound healer (Punshi, 1981). Broadly speaking, an aqueous extract of human placenta has the following actions in the body: it accelerates cellular metabolism providing the energy for the inflammatory response to occur. It also aids in absorption of exudates by controlling its formation, removal of unhealthy tissue by debridement and management of bacterial load that are required for good wound bed preparation (Hong et al., 2010). It stimulates tissue regeneration processes. Aqueous extract of placenta contain nucleotides like PDRNs and NADPH that are known for their regeneration effect (Nelson and Cox, 2000). In addition, it also supplements growth factors and small peptides that help in matrix formation and cell adhesion, thereby promoting wound healing as described in details later on.

The hydroalcolic extract of placenta is more effective for the treatment of vitiligo. The method of preparation of a hydroalcoholic extract of fresh human placenta meant for vitiligo treatment has been reported earlier (Pal et al., 1995). Such an extract from human placenta with efficient skin pigmenting activity has been developed based on experimental therapies. Glycosphingolipids, capable of inducing adhesion, spreading and motility of melanoma is present in the extract and therefore, may lead to skin pigmentation through induction of melanocytes (Pal et al., 2002).

3. Role of placental extract in wound healing

Wound healing is a very complex process that includes inflammation, cell migration, extracellular matrix deposition and cell maturation (Diegelmann and Evans, 2004). Several cytokines and growth factors are involved in inducing different cell types for healing. Extracellular matrixes such as collagen, laminin, and fibronectin are deposited in the dermis under the effects of fibroblasts in the later phase of healing. In the last phase, contraction of the dermis in full-thickness wounds and squamous differentiation of the keratinocytes on the surface of wounds concludes its maturation (Falanga, 2001). Among all the extracts of placenta that have been prepared, only the aqueous extract has been shown to have potent clinical efficacy in terms of healing. Aqueous extract from placenta is used as a licensed drug for wound healing under different trade names in India and overseas countries (Datta and Bhattacharyya, 2004a). This could be due the fact that the aqueous extract is a rich source of various bioactive peptides with tissue regeneration potential (Chakraborty and Bhattacharyya, 2005a; De et al., 2009). In addition, the extract also retains amino acids, nucleotides, polydeoxyribonucleotides and carbohydrates that might be responsible for wound healing. Most of the wound healers that are available in the market have anti-

microbial (antibacterial or antifungal) properties but the potency of the extract lies in the fact that it not only reduces the inflammatory phase and microbial burden on the wound (Chakraborty and Bhattacharyya 2005b) but also help in cell migration (Gupta and Chottopadhyay, 2008), matrix formation and tissue regeneration thereby ensuring sequential uninterrupted healing. Placental extract plays a beneficial role as a topical agent in the management of chronic non-healing wounds. It is reported that the extract promotes fibrogenesis, neoangiogenesis and epithelialisation (Shukla et al., 2004). Globally, the extract is now accepted as an effective healer in burn injuries, chronic non-healing wounds, post surgical dressings, as well as bedsores (Chakraborty et al., 2009).

Clinical evaluation of the aqueous extract revealed that it has anti-inflammatory and anti-platelet aggregation activity. As reported the extract exhibits anti-inflammatory response probably either through inhibition/inactivation of chemical mediators or by directly modulating prostaglandin (PG) production by suppression of cyclooxygenase (COX). Kinins, chemical mediators of nonimmunological type of inflammation, have two membrane receptor B1 and B2, for their activities. It has been reported that in cotton pellet induced subacute inflammation model, the extract may act as inhibitor of the B1-receptor thereby exerting its anti-inflammatory effect. It also helps in activation of the clotting cascade by trauma which results in platelet activation, followed by aggregation. The clinical study of platelet aggregation reflects that this extract can either inhibit PGs synthesis pathway or 5-hydroxytryptamine (5-HT) release. In addition, an aqueous extract of human placenta has also shown to stimulate collagen synthesis *in vivo* in rats. The significant increase in tensile strength and tissue DNA in the animals given the extract (intra muscular) indicates it was associated with marked collagen synthesis. The cytoplasmic repairment was revealed from regeneration of protein in appreciable amount. The efficiency of formation of collagen depends mainly on the synthesis of hydroxyproline, which was also shown to be appreciably high in the i.m. treated rats with the extract. These evidences, as reported, were further supported by pictures of histopathological changes showing maximum accumulation of collagen fibrils and epithelialisation (Biswas et al., 2001).

Human and animal models show that placental extract has an immunostimulating action both at cellular and humoral levels. It probably increases IgG and IgM at the humoral level and total lymphokines at the cellular level. It also reports several advantages over antibiotics and chemotherapeutic agents in terms of antibacterial activity including vascularisation of wound environment and is free from side effects (Chakraborty et al., 2009).

The injectable form of placental extract was found to be very effective, inexpensive and excellent stimulant of granulation tissue, which is superior to the dressing of povidone iodine (Pati et al., 2001). Human placental dressing was found to be effective in clinical wound healing for chronic varicose ulcers (Burgos et al., 1989). Later, this finding was supported by Subramanian et al., 1990. The wound healing potency of the aqueous extract of placenta is thus clinically well established and a lot of evidences support these findings. However, the active components present and the molecular mechanisms that are involved in the healing process are yet to be defined. The variety of biological actions of aqueous extract of human placenta is a matter of increasing interest. Characterization of such extract by isolating the active components present in it and to determine their mechanism of action related to wound healing process is of utmost requirement. This is because scientific

assessment of such extract is necessary for its better acceptance in medical practice with a more convincing approach.

4. Biochemical characterization and possible mechanism of action of the extract

Human placental extract manufactured by a proprietary extraction method using term human placenta as a raw material has potent therapeutic efficacy. Use of placental extract in different preparations under various trade names is a recommended worldwide practice. Developed from folk knowledge, the aqueous extract is used as a globally accepted licensed drug in post surgical dressings, as a healer in burn injuries and chronic wounds under different trade name in many countries including India (Chakraborty et al., 2009).

Isolating active components present in a biological extract is a natural propagation towards characterization of the drug. The contents present in the extract depend on the method of its preparation. An aqueous extract of placenta contains amino acids, peptides, glycosaminoglycans, lipids, polynucleotides (mainly polydeoxyribonucleotide fragments), vitamins, minerals etc. Some of them can be chemically or biologically synthesized but many of them can only be obtained by isolating them from a natural source. An aqueous extract of human placenta of potent therapeutic value is under research in terms of identifying the active components present and the biochemical basis of its mode of action. It was initiated at Indian Institute of Chemical Biology, Kolkata, India from 1999 and is still ongoing. The drug 'Placentrex' - manufactured by Albert David Ltd., Kolkata has been used for this purpose, which is used as wound healer from long back. The extract is prepared under relatively harsh conditions; as a result of which macromolecules are degraded generating low molecular weight bio-organic compounds e.g. amino acids, peptides, small sized polypeptides, nucleotides, small polynucleotide fragments etc. Owing to the presence of PDRNs, the spectral pattern of the extract corresponds mostly to that of DNA with a high absorption at 260 nm. Characterization of such extract by isolating the active components present in it and to determine their mechanism of action related to wound healing would be significant.

Major findings with its constituents include demonstration of minimal batch variation of the extract using conventional spectroscopic and chromatographic techniques like UV-VIS absorption spectra, FT-IR, TLC, HPTLC and HPLC and also by newly developed method for fingerprinting of multi-component drugs using fluorescence Excitation-Emission-Matrix (EEM) plots. Since there is no fingerprinting technique, which could be applied universally to separate different class of components, combination of different modes is used and upgradation of finger printing procedures is an active area of present research. Fluorescence spectroscopy has versatile application in chemistry and biochemistry. Modern versions of spectrofluorimeters are equipped with generation of three dimensional (3D) contour plots, also called EEM plots. where the z-axis represents emission intensity. Its advantage lies on presentation of chromophores having different excitation and emission patterns in a single profile. In analytical chemistry, it is frequently used in environmental and hydrological studies. Moreover, since wide excitation and emission zones are covered in a single profile, there is no scope of missing any newly emerged fluorophore in a batch. This safeguard is almost impossible to include in two-dimensional fluorescence emission profiles. A high

degree of consistency between different batches of the drug was observed from EEM. Validity of these results from EEM was crosschecked by different spectroscopic and chromatographic modes (Datta and Bhattacharyya, 2004b). This consistency reflects standardization of the manufacturing process of the drug and also adherence of uniform placental composition irrespective of the nutritional status of mothers. Fluorescence spectroscopy has been shown to have capability of identifying the fluorophores in a complex multi-component biological system.

During characterization of the extract, a fluorophore has been detected which had excitation and emission properties similar to nicotinamide adenine dinucleotide, reduced form (NADH) and nicotinamide adenine dinucleotide phosphate, reduced form (NADPH). When excited at 340 nm, it results in fluorescence emission having maxima around 436 nm, which is fairly specific for NADH and NADPH. These are natural constituents of placenta and play important roles in different metabolic pathways including wound healing. NAD/NADH and NADP/NADPH are major redox-active 'electron storage' compounds. One or both of these 'redox pairs' is involved in every major biochemical pathway. They participate in the trafficking of electrons as 'reducing equivalents,' the electron packets that facilitate metabolism. Evidence of only free and bound NADPH (supplying as a substrate or cofactor for enzymes) were demonstrated using thin layer chromatography and reversed-phase HPLC. Since NADPH is known to regulate a number of phenomena related to wound healing, we have investigated its presence in different batches of the drug. Biological functionality of the fluorophore in the extract has been confirmed by enzymatic assay. It has been shown that NADPH in aging human skin cells increases synthesis of collagen involving filaggrin and keratin *in vitro* (Datta and Bhattacharyya 2004a).

The extract also has the ability for *in vitro* NO induction in mouse peritoneal macrophage and human peripheral blood mononuclear cells (PBMC). The aqueous extract has been investigated in terms of induction of NO by macrophages. It has been demonstrated that with an increase of NO production, concomitant decrease in NADPH present in the applied placental extract was observed, thereby indicating that the NADPH pool of placental extract is metabolized, further justifying the biological potency of this nucleotide in the extract (Chakraborty et al., 2006). It has been well documented that nitric oxide (NO) has multiple effects at the molecular, cellular and physiological level in wound healing. It promotes inflammatory mediation of repair mechanism and wound matrix development followed by remodeling. NO mediated cellular signaling possibly enhances wound repair by increasing tissue oxygen availability through angiogenesis. It is produced in macrophages by the enzyme inducible nitric oxide synthase (iNOS) during wound healing (Luo and Chen, 2005). It acts as a biological signaling and effector molecule capable of diffusing across membranes and reacting with a variety of targets. Once induced, production of NO within the tissue can induce an environment that is toxic to invading microorganism (Efron et al., 2000). It promotes inflammatory mediation of repair mechanism and wound matrix development followed by remodeling. The extract has also shown to induce interferon- γ (IFN- γ) production by macrophages (Chakraborty et al., 2006).

Anti-microbial property of the extract against a large number of pathological microorganisms related to prevention of secondary infections to wounds has also been demonstrated. The extract has an effective inhibitory role on the growth of different microbes particularly growth of clinically isolated bacteria, e.g. *E. coli* from urine and blood culture and *S. aureus* from pus

cells. Drug resistant strains such as *E. coli DH5 α Pet-16 AmpR and Pseudomonus aeruginosa CamR* were also significantly inhibited by the extract. The extract has both bacteriostatic and fungistatic activities. Dose-dependent response of the extract was observed. A mixture of polydeoxyribonucleotides (PDRNs) appears to be the causative agent. Partial protection of the wound from secondary microbial infection is thus indicated. Though the mechanism of such growth-inhibitory activities has not been studied, it is predicted that the PDRNs present in the extract enter the microbes and interfere with their replication machinery (Chakraborty and Bhattacharyya, 2005b).

An important finding was isolation and purification of a peptide of around 7.4 kDa from the extract. Derived partial amino acid sequence from mass spectrometric analysis showed its homology with human fibronectin type III. Under nondenaturing condition, it formed aggregate, the elution pattern of which was identical to that of fibronectin type III as confirmed by reverse-phase HPLC. Immuno-blot of the peptide showed strong cross reactivity with reference human fibronectin type III-c. It draws special attention because its partial amino acid sequence showed homology with 10th type-III fibronectin peptide that also contains the 'RGD' signature sequence endowed with cell adhesion properties (Nath and Bhattacharyya, 2007). The importance of fibronectin in cutaneous wound healing is well documented as a general cell adhesion molecule by promoting the spread of platelets at the site of injury. It also helps in the adhesion and migration of neutrophils, monocytes, fibroblasts and endothelial cells into the wound region, and the migration of epidermal cells through the granulation tissue (Chakraborty and Bhattacharyya, 2005a).

Considering the importance of peptides in wound healing, the fraction was further characterized in terms of regulation of some enzyme activities related to repair mechanism. Primary investigation revealed that the drug stabilizes some serine proteases against their autodigestion by reversibly inactivating them, which enhances the efficiency of proteolytic enzymes thereby facilitates wound healing. Further it has been demonstrated that one or more peptides from human placental extract including fibronectin type III stabilize trypsin activity after strong association, which is reversible in nature. In presence of excess substrate, the conjugate is dissociated. Regulation of trypsin activity with prevention of autodigestion has been demonstrated in De *et al*, 2011.

As a working hypothesis, wound healing can be broadly categorized into three overlapping phases both in terms of time and space — cleansing or debridement, following which proliferation occurs to provide a platform for tissue regeneration and finally differentiation occurs. During debridement, extensive 'hydrolytic activity' ensures proper cleaning of the wounded tissue. The last two stages of healing require extensive 'synthetic activity' and minimal hydrolytic activity. Trypsin and similar proteolytic enzymes help in debridement and prevent keloid formation during wound healing and therefore regulation of its activity is an important criterion. Trypsin, chymotrypsin, collagenase, papain, bromelain etc were reported to be effective in wound healing as a debriding agent. These agents remove foreign bodies and necrotic tissues and reveals healthy, bleeding tissues so that the wound can heal (Buck and Phillips, 1970; Craig, 1975; Ramundo and Gray, 2009). Thus, it is expected that the peptide would have greater half-lives in the blood, as serine proteases form a major part of blood proteases. Proportionate mixing of 'Placentrex' with some proteolytic enzymes and subsequent evaluation of its efficacy in wound healing, may be another promising avenue of the future study leading to the development of an effective wound healer with debridement potential.

It remains questionable whether the proteins and peptides of the drug remain stable in human blood as the blood contains many proteases. There is an array of proteases in blood, which include thrombin, plasmin, Hageman factor, blood coagulation cascade enzymes etc. Though it is well known that blood proteases are quite specific about their substrates, the question of stability of the drug components remains an important issue from clinical point of view. It has been demonstrated by size exclusion HPLC that the peptide fraction of the drug remains unaffected in presence of plasma and serum proteases from human blood (Fig. 1). In reverse, it has also been demonstrated by gelatin zymography that the blood proteases remain unaffected after incubation for 48 hrs in presence of the placental peptide fraction (Fig. 2). However, protease-substrate interactions are not always guided by the specificity of the protease. One essential requirement for proteolysis is that the hydrolysable bond must be physically accessible to the catalytic site of the protease. Often proteins can survive proteolysis out of its compact globular structures under physiological conditions in presence of proteases. It is generally observed that any drug administered through intra venous or intra muscular remains active in circulation for a maximum period of 48 hours after which it is removed from circulation by metabolism or excretion. Thus the studies were restricted within 48 hrs.

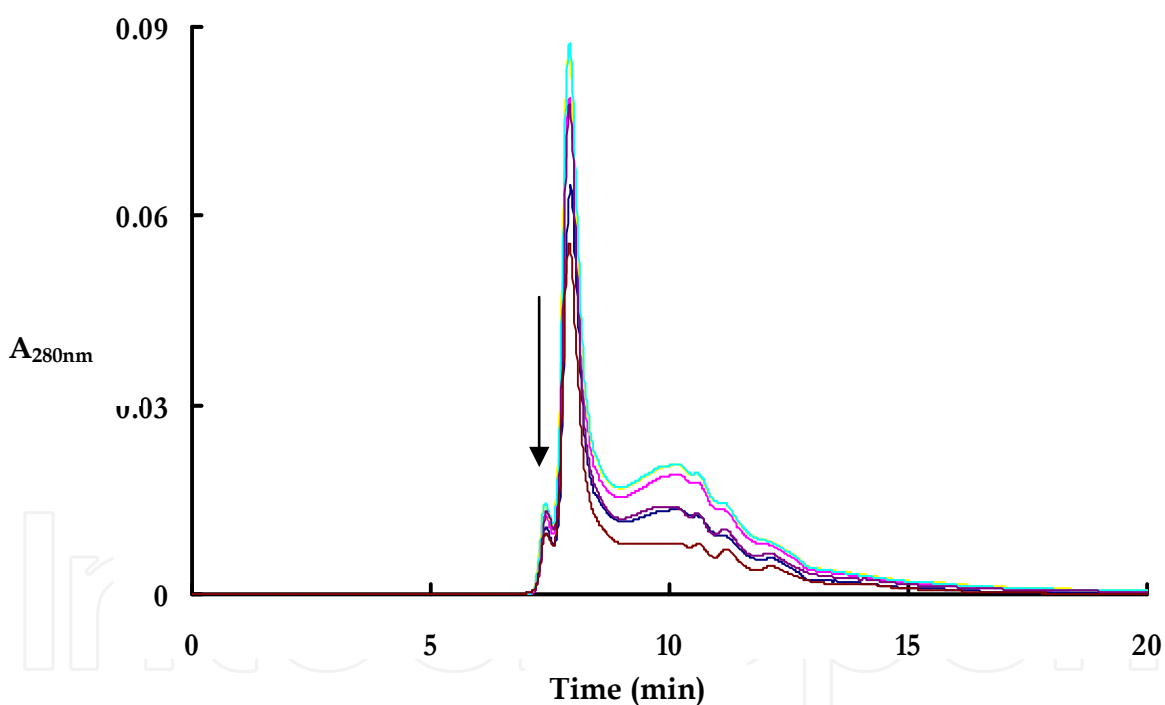


Fig. 1. Stability of the peptide fraction from placental extract in presence of blood protease (plasma) over 48 hr as observed from size exclusion HPLC using Waters Protein Pak 125 column (fractionation range 2-20 kDa). The sharp major peak corresponds to the unresolved peptide fractions of Mw 10-16 kDa while the second peak corresponds to elution of small bioorganic molecules. The overlapping profiles from top to bottom indicate samples of incubation time 0, 6, 18, 24, 48 hr respectively at 37°C. The loss of intensity appears to be due to partial precipitation of the peptides removed by centrifugation though there is no indication of multimer formation at the void volume of the column. The downward arrow indicates the void volume.

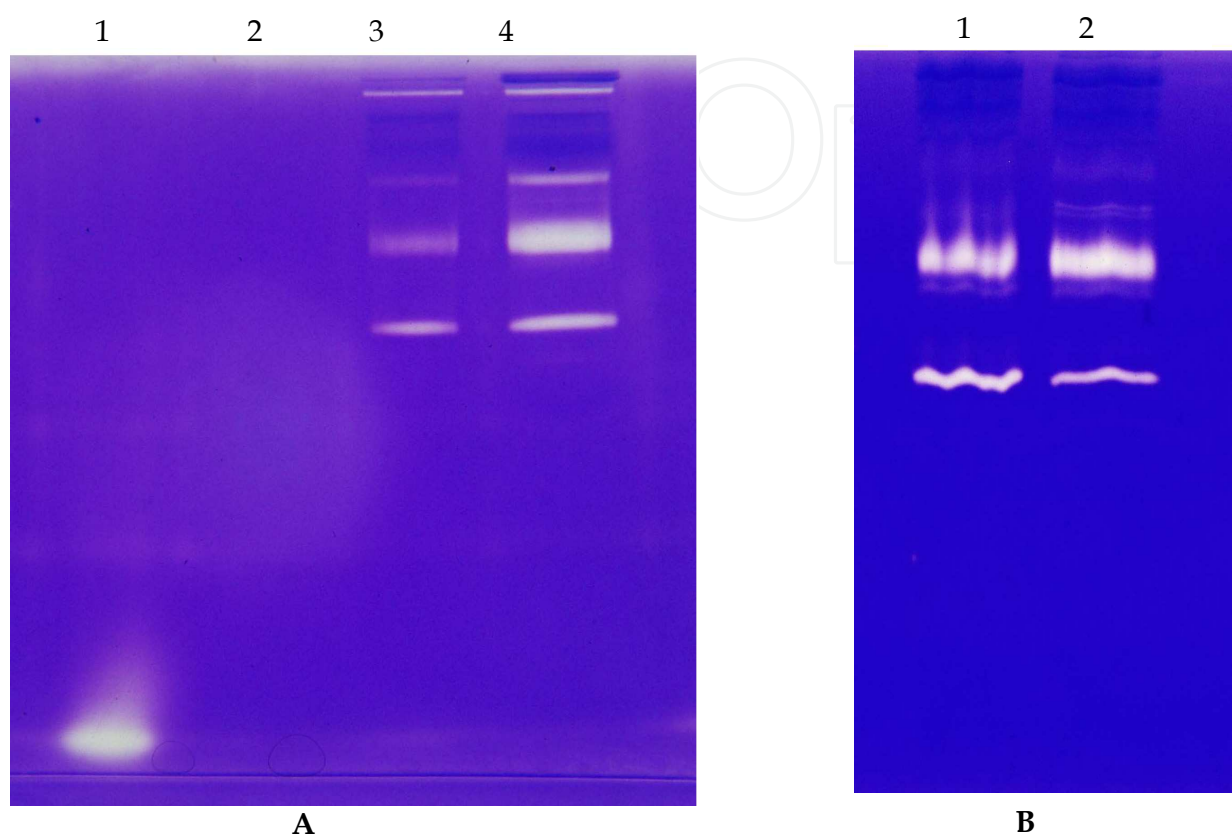


Fig. 2. Stability of blood proteases in presence of peptide fraction of human placental extract as observed by gelatin zymography. (A) Representing trypsin 0.75 ng (lane 1), buffer as control (lane 2), serum isolated from blood (lane 3) and plasma (lane 4), both were incubated for 48 hr with 10 μ g/ml of peptide fraction of the placental extract. (B) Human serum (lane 1) and plasma (lane 2) as control.

As a part of the healing process, the body enters a hypermetabolic phase, where there is an increase in demand for carbohydrates. Cellular activity is fuelled by adenosine triphosphate (ATP), which is derived from glucose, providing the energy for the inflammatory response to occur. In addition to their role in metabolic process, carbohydrates are involved in cellular signaling as well as cell-cell interaction. While addressing roles of carbohydrates, interaction of lysozyme with the extract was investigated. The enzyme showed positive reactivity in a time and concentration dependent manner indicating that carbohydrate residues were present. Attempts are in progress to identify these residues by GC-MS in presence of reference sugars (unpublished observation).

This multifaceted character of the drug encourages further investigation following which the biochemical basis of its mode of action is likely to be highlighted.

5. Multiple therapeutic properties of aqueous extract of human placenta

Several clinical investigations and findings have been reported on effective therapeutic use of placental extract such as clinical evaluation in radiation-induced oral mucositis (Kaushal et al., 2001), restorative effects in X-ray-irradiated mice (Mochizuki and Kada, 1982), for the treatment of myopic and senile chorio-retinal dystrophies (Giroto and Malinverni, 1982), rheumatic arthritis (Rosenthal, 1982), osteoarthritis (Kim et al, 2010), skin diseases (Punshi, 1981), for prevention of recurring respiratory infections (Lo Polito, 1980), in the therapy of urticarias (Mittal, 1977), in therapy of children with asthmatic bronchitis (Vecchi et al., 1977), in atrophic rhinitis (Sinha et al, 1976), on hepatic drug metabolizing enzymes (Bishayee et al., 1995), in cerebral arteriosclerosis (Lafay 1965), in periodontal disease (Calvarano et al., 1989). Topical treatment of cervico-vaginal lesions using a placental extract with anticomplement activity (Sgro, 1978), local therapy of psoriasis (Lodi et al., 1986), treatment of vitiligo with topical melagenine (Suite and Quamina, 1991) has also been reported to be successful. A number of reports have been found on placental extract effective in arthritis (Yeom et al., 2003). Effectiveness of topical application of polydeoxiribonucleotide from human placenta in gynaecology has been documented (Bertone and Sgro, 1982). Post-irradiation cystitis improved by instillation of early placental extract in saline (Mičić and Genbacev, 1988) and placental extract injections in the treatment of loss of hair in women is also documented (Hauser, 1982). It was reported that aqueous human placental extract induced potentiation of morphine antinociception may have a clinical significance for the treatment of persistent or chronic pain (Gurgel et al., 2000). Clinical evaluation of the aqueous extract has shown that the drug acts as a potent wound healer with anti-inflammatory and immunotropic effect. Placental extract has long been used as a cosmetic supplement for skin care and skin pigmentation (Pal et al., 2002). Recently it has been reported that menopausal symptoms and fatigue in middle-aged women improved after the treatment with the aqueous placental extract (Kong et al., 2008). It has been reported that an aqueous extract of human placenta was found to offer protection against established experimental visceral leishmaniasis in BALB/c mice and hamsters, whether the *Leishmania donovani* strain involved was one that was sensitive or resistant to pentavalent antimony. Based on the results of this pilot study, a further evaluation of the efficacy of human placental extract therapy, which may offer a cost-effective way of improving the treatment of antimony-resistant cases of visceral leishmaniasis, is being undertaken (Chakraborty et al., 2008).

Several studies have shown therapeutic use of other animal placenta also. Recent report shows that the protective effect of porcine placental extract in contact hypersensitivity is mediated by inhibition of the inflammatory responses and IgE production to modulate skin inflammation (Jash et al., 2011). Cow placental extract efficiently accelerates cell division and growth factor expression by raising the insulin-like growth factor (IGF-1) mRNA and protein level to increase hair follicle size and hair length in murine (Zhang et al., 2011). Bovine placental extract is capable of improving the tenderness of certain injuries that are

relatively high in connective tissue, while avoiding myofibrillar protein hydrolysis (Phillips et al. 2000). An immunomodulatory peptide was isolated from aqueous extract of bovine placenta which showed no significant homology with other immunomodulatory peptides (Fang et al., 2007). Thus, the therapeutic potency of placental extract has earned global recognition.

6. Conclusion

Natural medicine continues to play an important role for prevention, alleviation and cure of diseases. In some part of the Western world, the use of traditional medicine has been largely lost. However, it is a widespread phenomenon in the developing countries where 80% of the population is still relying on traditional medicine for primary healthcare. Derived from folklore, human placental preparations show immense therapeutic value and can be safely used once it is ensured that the source is free from fatal infections like HIV, HBV, HCV and alike. The aqueous extract of human placenta is a scientifically proven potent wound healer. Characterization of active components present in different placental preparations and correlating them with their therapeutic actions are the promising avenue for future study. A fibronectin type III-like peptide present in the aqueous extract appears to be one of the key components for wound healing. It is known from the literature that this type of peptide inhibits tumor growth, angiogenesis and metastasis. Therefore, future work should include evaluation of human placental extract as anti-tumor agent. Identification of the possible signaling pathways for wound healing as well as other therapeutic properties of the placental peptide should receive immediate attention. Additional components identified include PDRN and NADPH. Further, yet unidentified peptides or small molecules might also be present in various preparations of the extract that might play roles in wound healing and related disorders. Identification of other biologically active components in the extract and their mechanism of action in terms of cellular signaling, which play significant role in wound healing also needs to be addressed.

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This book contains the total of 19 chapters, each of which is written by one or several experts in the corresponding field. The objective of this book is to provide a comprehensive and most updated overview of the human placenta, including current advances and future directions in the early detection, recognition, and management of placental abnormalities as well as the most common placental structure and functions, abnormalities, toxicology, infections, and pathologies. It also includes a highly controversial topic, therapeutic applications of the human placenta. A collection of articles presented by active investigators provides a clear update in the area of placental research for medical students, nurse practitioners, practicing clinicians, and biomedical researchers in the fields of obstetrics, pediatrics, family practice, genetics, and others who may be interested in human placentas.

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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