Received: 11 July 2018 | Revised: 28 February 2019 | Accepted: 16 March 2019

DOI: 10.1002/med.21579

WILEY

REVIEW ARTICLE

Bioactive peptides and proteins as alternative antiplatelet drugs

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Funding information
Science and Engineering Research Board, Grant/Award Number: PDF/2017/001166/LS

Abstract

Antiplatelet drugs reduce the risks associated with atherothrombotic events and show various applications in diverse cardiovascular diseases including myocardial infarctions. Efficacy of the current antiplatelet medicines including aspirin, clopidogrel, prasugrel and ticagrelor, and the glycoprotein IIb/IIIa antagonists, are limited due to their increased risks of bleeding, and antiplatelet drug resistance. Hence, it is important to develop new effective antiplatelet drugs, with fewer side-effects. The vast repertoire of natural peptides can be explored towards this goal. Proteins and peptides derived from snake venoms and plants represent exciting candidates for the

Abbreviation: AD, Alzheimer's disease; ADMET, absorption, distribution, metabolism, excretion, and toxicity; ADP, adenosine 5'-diphosphate; APTT, activated partial thromboplastin time; CVD, cardiovascular diseases; eNOS, endothelial nitric oxide synthase; Nva, norvalin; PTT, partial thromboplastin time; TNF-α, tumor necrosis factor-alpha; vWF, von Willebrand factor.

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development of novel and potent antiplatelet agents. Consequently, this review discusses multiple peptides that have displayed antiplatelet aggregation activity in preclinical drug development stages. This review also describes the antiplatelet mechanisms of the peptides, emphasizing the signaling pathways intervened by them. Also, the hurdles encountered during the development of peptides into antiplatelet drugs have been listed. Finally, hitherto unexplored peptides with the potential to prevent platelet aggregation are explored.

KEYWORDS

antiplatelet effects, bioactive peptides, cardiovascular disease, drug discovery, medicinal plants, pharmacokinetic profile

1 | INTRODUCTION

Platelets, or thrombocytes, are minute rough-shaped anucleate cell fragments, derived from progenitor megakaryocytes that circulate in the blood.[1] Platelets are vital for maintaining hemostasis. But in stress, they can undergo aggregation, adhesion, and procoagulant activation. Platelets have surface receptors and granules which determine their specific cellular identity. Upon vascular injury, platelets are exposed to a wide variety of extracellular proteins and are activated.[2] The resulting activation causes the platelets to undergo rapid morphological and biochemical changes that support aggregation. Platelets can change their phenotype by expressing receptors on their surface after activation. For example, P-selectin, a cell adhesion molecule, is expressed only on activated platelets. Platelet-secreted substances include serotonin, adenosine diphosphate (ADP), polyphosphates, hemostatic factors (Factor V, von Willebrand factor [vWF], and fibrinogen), growth factors (platelet-derived growth factor, basic fibroblast growth factor, and stromal cell-derived factor-1α), proteases (matrix metallopeptidase 2 such as MMP2 and MMP9), angiogenic factors (angiogenin and VEGF), anti-angiogenic factors (e.g. angiostatin and PF4), necrotic factors (e.g. tumor necrosis factors such as TNF-α and TNFβ), and other cytokines.[3–7] Substances recruit other platelets, causing more platelets to adhere to the subendothelial matrix, and to coalesce with one another at the site of injury, eventually forming a primary hemostatic plug.[8] Binding of fibrinogen to glycoprotein IIb/IIIa (GP IIb/IIIa) on stimulated platelets causes their aggregation.

Platelets are involved in the fundamental biological process of inflammation through the direct interactions with other cell types such as leukocytes and endothelial cells, via its releasates and secretomes.[9] However, dysregulated platelet activation translates into a wide spectrum of pathological conditions such as renal diseases, tumorigenesis, thrombotic diseases, including venous and arterial thrombosis, embolism, and stroke.[10,11]

Atherothrombosis represent a major global public health burden. Aggregation of platelets at the sites of atherosclerotic plaque can provoke vascular occlusive thrombi, resulting in acute coronary syndrome, stroke, transient ischemic attack, and critical limb ischemia.[12,13] In response to specific proinflammatory signals, endothelial cells become more adhesive towards platelets, triggering the secretion of various platelet-derived inflammatory molecules, that create a positive feedback loop that likely plays a role in further endothelial cell activation and platelets recruitment.[14] Endothelial cell-bound platelets are highly efficient at recruiting monocytes and macrophages from the circulating blood. It enhances the formation of platelet-monocytes aggregates, which are released to the site of the pro-inflammatory stimulus. Therefore, pathological derangement of these key

interactions among platelets, endothelial cells, and leukocytes facilitate the inflammatory process that contribute to the development of chronic atherosclerosis.[14,15]

Antiplatelet drugs have been established to reduce the risk of atherothrombotic events and to manage cardiovascular and cerebrovascular diseases.[16,17] The crucial step in both protective hemostasis and pathological thrombosis is platelet activation, which can occur via multiple pathways by the binding of specific agonists, such as thromboxane A2 (TxA2), ADP and thrombin, to their corresponding receptors on the platelet surface.[18,19] Others factors that can contribute to platelet activation include epinephrine, prostaglandin E_2, serotonin, and various chemokines.[20,21] However, these factors predominantly serve to potentiate platelet activation induced by other stimuli and their effect is very weak.[19,22] Current approved oral antiplatelet drugs target the TxA2 (aspirin) and ADP ($P2Y_{12}$ inhibitors such as clopidogrel, ticlopidine, and prasugrel) platelet activation pathways and have shown to significantly reduce the incidence of ischemic events in patients suffering from atherothrombotic disease.[19,23] Another class of antiplatelet agents include the GP IIb/IIIa receptor antagonists which mediate their activities via blockade of the GP IIb/IIIa receptor, is involved directly in the binding of fibrin and allows the aggregation of adjacent platelets.[24] The three GP IIb/IIIa receptor antagonists currently available for clinical use are eptifibatide, tirofiban and abciximab.[25] Aspirin irreversibly inhibits cyclo-oxygenase-1 (COX-1), an enzyme responsible for the formation of prostaglandin, thereby inhibiting the synthesis of TxA_2, an important platelet activator,[15] while ADP receptor pathway inhibitors such as clopidogrel and ticlopidine irreversibly inhibit the ADP dependent pathway of platelet activation, by covalently modifying and inactivating the platelet P2Y(ADP) receptor (also called $P2Y_{12}$), which is physiologically coupled to the inhibition of adenylyl cyclase.[8,26] The efficacy of aspirin and clopidogrel is well documented and the dual antiplatelet therapy has emerged as the standard of care in acute coronary syndromes. Typically, aspirin is used in combination with a $P2Y_{12}$ inhibitor.[15,19]

However, despite the established benefits of aspirin and ADP receptor inhibitors, these agents have clinical limitations, which include the increased residual risk for ischemic events, bleeding, and variable inhibition of platelet aggregation.[27,28] Bleeding is directly or indirectly associated with myocardial infarction and stent thrombosis. The impact of antiplatelet drugs on bleeding varies. The most frequent hemorrhagic complication associated with aspirin is gastrointestinal bleeding, which occurs due to the peculiar mechanism of action of aspirin. It inhibits COX-1 enzyme, which plays a protective role against ulcers by producing gastroprotective PGE_2 in the stomach. This detrimental effect of aspirin is dose-dependent.[29,30] Furthermore, several patients

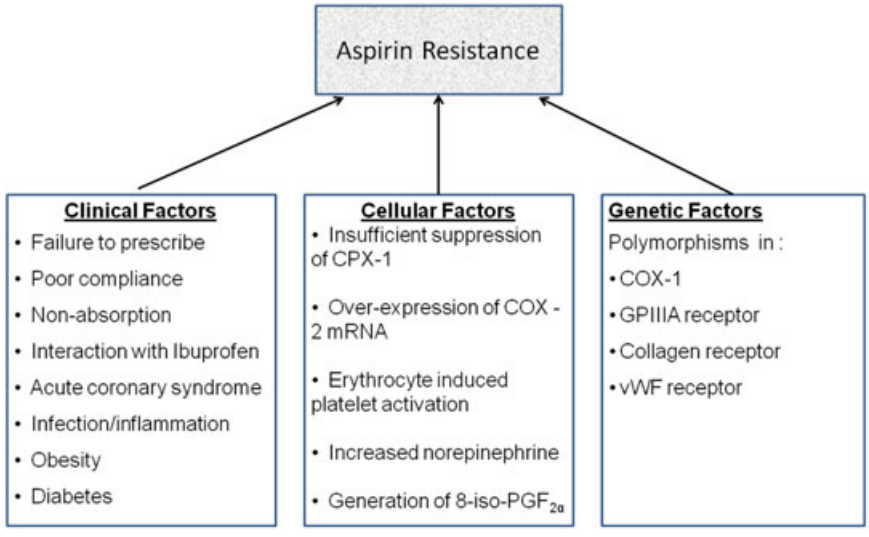

FIGURE 1 Mechanisms of aspirin resistance. COX1, cyclo-oxygenase-1; GPIIIA, glycoprotein IIIa; mRNA, messenger RNA; PGF, platelet growth factor; vWF, von Willebrand factor [Color figure can be viewed at wileyonlinelibrary.com]

do not respond appropriately to these agents by unknown mechanisms, which is a form of "antiplatelet drug resistance" (Figure 1). The inefficacy of these antiplatelet agents can arise from variations in pharmaceutical preparations, drug absorption and metabolism, drug interactions, high platelet turnover, medication adherence, environmental/lifestyle factors, and the modifications of a drug's therapeutic target.[31,32] Resistance to the most common and active antiplatelet agent, aspirin, has also been demonstrated.[31–35] These individuals are most prone to acute cardiovascular events, and the validated clinical approaches for these patients are very scarce. Large-scale randomized multicenter clinical trials testing platelet function have failed to demonstrate clinical results with individualized antiplatelet therapy.[36–38] Similarly, gender differences have also been explored in the effective treatment of cardiovascular complications that require antiplatelet agents.[21] These facts demonstrate that there is a pressing medical need for novel antiplatelet agents with a more favorable safety profile.

Peptides have recently gained significant attention as potential therapeutic agents for treating a plethora of ailments.[39–46] Over 7000 naturally-occurring peptides have essential roles in modifying human pathophysiology, falling under groups such as growth factors, anti-infective agents,[47] hormones, ion channel ligands,[48] and neurotransmitters.[49] They represent a unique class of pharmaceutical compounds, molecularly poised between small molecules and proteins, but with different biochemical and biological properties as compared to both. Unlike small molecule drugs, peptides represent only 2% of the worldwide drug market.[50] Peptides have high target affinity, specificity and potency, show minimum toxicity since their by-products are amino acids and have a short half-life.[51] This preference is reflected in data, with approximately 140 peptides under evaluation for therapeutics at present.[52] It is notable to mention that about 60 approved peptides by US Food Drug Administration are available for the treatment of various disorders, and above 500 peptides are in the preclinical development stage.[53] Despite their favorable properties, peptides have some intrinsic limitations, including their poor bioavailability and interaction of peptide food matrices during the development process, which have declined their importance. Therefore, particular attention needs to be attributed to these parameters in the preparation of peptides.[54] Numerous recent studies have illuminated that peptides exhibit antithrombosis and antiplatelet aggregation activities.[55–57] This review summarizes the potential of peptides from natural sources, to exhibit antiplatelet aggregation activity. Additionally, it describes the possible mechanisms-of-action through which they exert their antiplatelet effects (Figure 2).

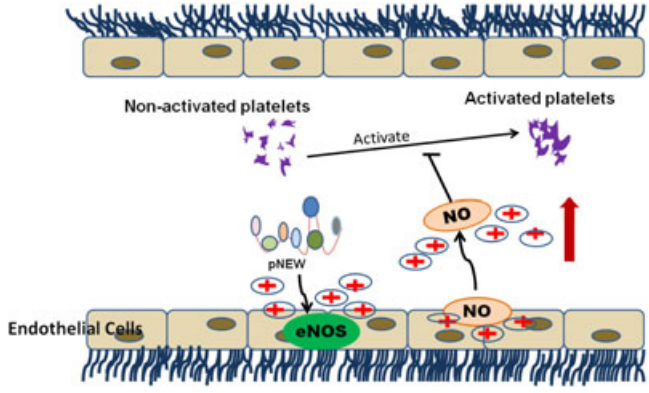

FIGURE 2 pENW (pGlu-Asn-Trp) promotes NO generation from vascular endothelial cells by increasing the expression and activity of eNOS. NO synthesized by the endothelial cells potentiates the antiplatelet effect of pENW. eNOS, endothelial nitric oxide synthase; NO, nitric oxide [Color figure can be viewed at wileyonlinelibrary.com]

2 | STRATEGIC APPROACHES TO OPTIMIZE PHARMACOKINETIC PROFILE OF PEPTIDES

Natural peptides usually have a weak pharmacokinetic profile with poor absorption, distribution, metabolism, and excretion (ADME) properties with short half-life and rapid clearance. With a few exceptions (eg, cyclosporine, a lipophilic cyclic peptide with 11 amino acids), most peptides have less than 1% oral bioavailability. Strategies to enhance druggability of peptides include increasing penetration and half-life while reducing proteolysis and renal clearance.[58] To evaluate ADME properties, various in vitro, in vivo and in silico tools, in addition to structural modifications are used to improve peptide druggability.[58] Currently, protein molecules and therapeutic peptides are administered parenterally (subcutaneous, intramuscular, or intravenous) and those which require frequent administration result in poor patient compliance. However, several formulation approaches (proteinylation, glycosylation, and PEGylation) and physical approaches (absorption enhancers and metabolism modifiers) may be used to facilitate the oral delivery efficiency of proteins and peptides. Chemical modification early in the drug-discovery process strategies involve producing an altered chemical entity with improved physicochemical properties (solubility, permeability, and stability) along with its biological properties such as selectivity or affinity toward its target receptor which the physical strategies comprise formulation-based approaches including application of chemical superior excipients (absorption enhancers and enzyme inhibitors), or a colloidal carrier system (nanoparticles, microspheres, liposomes, micro-, and nano-emulsions).[59] Peptides have very short biological half-lives in vivo due to their rapid digestion in gastrointestinal system by proteolytic enzymes, protein-modifying chemicals or through other clearance mechanisms. Therefore, peptides have uncertain therapeutic potential, which leaves room for several question marks in the process of drug design.[60]

Limiting enzymatic breakdown by the identification of cleavage sites, succeeded by the replacement of specific amino acids, is one of the first approaches in extending plasma half-life of peptides. Other techniques include enhancement of the secondary structure (folding), addition of structures inducing probe tail (SIP), stapling or clipping of peptide sequences,[44] and cyclization[45] or lactam bridges.[61] Modification of the N- or/and C-termini usually improve peptide stability. For instance, the peptide tesamorelin has much longer half-life (1 hour) than the natural growth hormone-releasing hormone (6.8 minutes) due to a hexenoyl group attached to the N-terminus tyrosine residue.[62] Similarly the stapled ALRN-5281, is a proprietary agonist for treating orphan endocrine disorders, is currently in a clinical trial for treating orphan endocrine disorders.[58,63] Binding peptides to albumin protein can relax dosage frequency down to once a week by means of techniques such as peptide acylation (GLP-1 agonist),[64] conjugating antibody fragments that bind to albumin and insertion of albumin binding fragments into the peptide backbone.[65] The repertoire of available peptides thus needs to be sieved for novel antiplatelet agents, so as to counter drug resistance.

Diverse natural novel proteins and peptides which affect cardiovascular physiology can be used in controlling various pathological events resultant of platelet aggregation and clotting disorders[66–70] (Table 1). All these pathologies are studied in same experiments, as they are interrelated and a ligand affecting one parameter also influences others, to an extent providing synergistic action.[34,78] Peptides which have demonstrated antiplatelet activity in preclinical studies are discussed below.

3 | PRECLINICAL EVIDENCE OF ANTIPLATELET PEPTIDES

3.1 | Peptides from snake venom

Snake venom liquor or snake wine are used for the prophylaxis and rehabilitation of patients with cardiovascular disease in Traditional Chinese Medicine (TCM). A study was conducted to investigate the antiplatelet aggregation efficacy of two trigopeptides, Pt-A (Glu-Gln-Trp) and Pt-B (Glu-Asn-Trp), isolated and sequenced from venom liquor

TABLE 1 Peptides with their source, additional pharmacological effects, and mechanisms of actions.

Peptide/source	Antiplatelet aggregation IC_{50}, μM	Additional pharmacological effect	Mechanism of action	References
EQW *Deinagkistrodon acutus* venom	203	Antithrombotic activity, low hemorrhagic risk	–	[56]
LTFPRIVFVLG *Agkistrodon acutus* venom	204.24	Low hemorrhagic risk, inhibit factor Xa	–	[57]
(L1α) GDNKPPKKGPPNG *Vipera lebetina* venom	0.0023	–	Inhibit fibrinogen binding to platelets	[98]
(L1β) DNKPPKKGPPNG *Vipera lebetina* venom	0.0025	–	Inhibit fibrinogen binding to platelets	[98]
(L1γ) NKPPKKGPPNG *Vipera lebetina* venom	0.003	–	Inhibit fibrinogen binding to platelets	[98]
GPRP Fibrinogen (natural platelet–adhesive protein)	70	–	Inhibit fibrinogen binding to platelets	[98]
LGGAKQAGDV γ Chain of fibrinogen (natural platelet–adhesive protein)	50-100	–	Inhibit fibrinogen, fibrin and von Willebrand factor binding to platelets	[71]
RGDS Fibronectin (natural platelet–adhesive protein)	15	–	Inhibit fibronectin binding to platelets	[72]
GK-(Hyp)-GE-(Hyp)-GPK Collagen peptide α_1(III)CB4 (natural protein)	–	–	–	[99]
KPGEPGPK Type III collagen (natural protein)	–	–	–	[82]
RQMIRGYFDV Murine monoclonal antibody "AC7" (mouse)	700	–	Inhibit fibrinogen binding to platelets	[73]
AYADUALIN *Lutzomyia ayacuchensis* (insect)	5.66	Prolong APTT but not PT; inhibit kallikrein, factor IXa, Xa and XIIa blocking the intrinsic pathway of coagulation	Inhibit binding of $\alpha_{IIb}\beta_{III}$ to fibrinogen	[74]
FRGCWLKNYSPRGCL-NH_2 *Amolops loloensis* (frog)	12.27 μg/mL	–	–	[75]
Ixorapeptide I *Ixora coccinea* (plant)	–	–	–	[76]
Ixorapeptide II *Ixora coccinea* (plant)	–	–	–	[76]
PS-(Nva)-GDW (synthetic)	0.46	–	Specific inhibitor of $\alpha_{IIb}\beta_{III}$; Inhibit fibrinogen binding to platelets	[100]

(Continues)

TABLE 1 (Continued)

Peptide/source	Antiplatelet aggregation IC_{50}, μM	Additional pharmacological effect	Mechanism of action	References
IPRGDMPA (modified sequence present in disintegrin, from snake venom as synthetic	2.2-4	–	Interfere in the interaction between fibrinogen and its receptor	[85]
Z4A5 (synthetic)	0.21-046	–	Binds to glycoprotein IIb/IIIa	[100]
Octapeptide Lys-Pro-Gly-Glu-Pro-Gly-Pro-Lys (synthetic)	100% inhibition at 2 mM	–	Inhibition of type III collagen-induced platelet aggregation	[82]
Oat hydrolysates (dietary)	Glutelin 0.315 mg/mL Albumin 0.292 mg/m:	–	COX-1 inhibition thereby prevents Thromboxane A2 formation	[77]
Buckwheat hydrolysates (dietary)	Glutelin 0.326 mg/mL Albumin 0.897 mg/mL	–	COX-1 inhibition thereby prevents Thromboxane A2 formation	[77]
Barley hydrolysates (dietary)	Albumin 0.897 mg/mL	–	COX-1 inhibition thereby prevents Thromboxane A2 formation	[77]

of *Deinagkistrodon acutus* (sharp-nosed pit viper). The antiplatelet activity of the tripeptides evaluated using ADP-induced platelet aggregation assay revealed that both peptides significantly inhibited platelet aggregation, with IC_{50} values of 0.066 mM (Pt-A) and 0.203 mM (Pt-B).[56] These results demonstrate that Glu-Asn-Trp and Glu-Gln-Trp could prohibit the aggregation of platelets and thrombus formation, without increasing the risk of hemorrhage.[56]

Emerging evidence has established that bioactive peptides produced as a result of pro-enzymatic hydrolysis, exhibit a better absorption profile and have a versatile characteristics. One such peptide is ACH-11, derived from the hydrolysate of *Agkistrodon acutus* venom, with an amino acid sequence LTFPRIVFVLG. This peptide is an inhibitor of both Factor Xa and the aggregation of platelets. Factor Xa is a key component of the prothrombinase complex that converts prothrombin to thrombin. ACH-11 inhibits the catalytic function of Factor Xa and platelet aggregation, without serious bleeding risk.[57]

The peptides of the lebetin 1 family have emerged as potentially useful antiplatelet agents. *Vipera lebetina* venom peptide lebetins can be of variable length. Short lebetin 1 include L1α [GDNKPPKKGPPNG] and L1β [DNKPPKKGPPNG] while long lebetin 2 include L2α [GDNKPPKKGPPNGCFGHKIDRIGSHSGLGCNKVDDNKG] and L2β [DNKPPKKGPPNGCFGHKIDRIGSHSGLGCNKVDDNKG]. L1α and L1β were found to inhibit the aggregation of platelets, with IC_{50} values of 2.3 and 2.5 nM, respectively. L1 peptides (0-50 μg/kg body weight dissolved in 0.9% NaCl) elicited no toxic effects when injected intracerebroventricularly, intraperitoneally, or subcutaneously in mice.[65] Like the snake venom peptides, the antiplatelet properties of proteins from snake venom have also been studied in animals. The snake venom has small proteins called disintegrins. The disintegrin members such as echistatin, flavoridin, albolabrin, applagin, barbourin, obtustatin, schistatin, batroxostatin, bitistatin, elegantin, eristicophin, and kistrin have demonstrated consequent antiplatelet action in different animal models such as buffalo, dog and horse. The overall

effect was species-dependent, with kistrin being up to 1.6-fold more efficient compared to flavoridin, in inhibiting of ADP-enhanced platelet aggregation in both dogs and buffalo. In contrast, flavoridin exhibited 2.1-fold stronger effects than kistrin while inhibiting the binding of platelets from horses.[66] Arg-Gly-Asp (or RGD), the cell recognition signal, was found to potently inhibit the aggregation induced by ADP in both human and canine platelet-rich plasma. Nonetheless, there was distinct specificity in the effects, depending on the species tested.[67]

3.2 | Endogenous proteins and peptides as platelet inhibitors

Some peptides have amino acids with side chains which can interfere with the fibrinogen binding to the platelets. These peptides have homologous motifs in the fibrinogen. Such peptides include γ-chain peptides, Gly-Pro-Arg-Pro, and Arg-Gly-Asp, among others.[68,69] While Gly-Pro-Arg-Pro exhibits homology to sequences in fibrinogen, Arg-Gly-Asp sequences can be seen in fibrin and von Willebrand Factor (vWF) as well, apart from two positions in fibrinogens. The γ-chain peptides and Arg-Gly-Asp can antagonize the interaction of adhesive proteins with platelets.[70,78] Another peptide, Arg-Gly-Asp-Ser, inhibits the binding of fibronectin to platelets (IC_{50} values of approximately 10-20 μM).[79] The first three amino acids are the critical players in the inhibition. Gly-Pro-Arg-Pro peptides can selectively inhibit the binding of fibrinogen to platelets.[80] Gly-Pro-Arg-Pro can inhibit fibrinogen binding to the thrombin-stimulated platelets, in a dose-dependent manner. A 50% inhibition of fibrinogen binding occurred at a 70 μM concentration.[72] Gly-Pro-Arg-Pro peptides are not directly involved with the binding of fibrinogen to platelets, the peptide is perhaps an antagonist which binds to the ligand, preventing its interaction with receptors.[72,80,81] A decapeptide, LGGAKQAGDV, which corresponds to the residues 402 to 411 of the fibrinogen γ-chain, inhibits the binding of fibrinogen to the thrombin-stimulated platelets. Fibrinogen binding of the peptide depended on the applied dosage, where the optimal dose has IC_{50} of 50 to 100 μM and maximum inhibition above 90%. This peptide prevents the binding of fibronectin and vWF platelets stimulated by thrombin. Gamma chain peptides inhibit fibrinogen binding at equilibrium and with equal potency in the presence of magnesium and calcium.[71,76,82]

Bradykinin is a short-lived vasoactive peptide that has been reported to promote vasodilation, exerts antiproliferative effects and inhibits thrombin-induced platelet activation in vitro.[72,80,81] The stable metabolic end-product of bradykinin, is a pentapeptide, known as bradykinin (1-5) which is formed by the proteolytic action of angiotensin-converting enzyme. The study carried by Murphey et al[72] have demonstrated that Bradykinin (1-5) has the ability to inhibit platelet aggregation in humans through a novel mechanism without causing vasodilation

Apelin peptide is the endogenous ligand of APJ, a G protein-coupled receptor. The apelin/APJ system is involved in a number of physiological and pathophysiological conditions[79] and altered apelin/APJ concentration are associated with aortic valve stenosis,[71] atherosclerotic coronary arteries,[82] acute myocardial infarction and angina.[76,83] Adam et al[84] investigated into the effect of apelin on platelet function and the results showed that apelin was found to mainly inhibit thrombin- and collagen-mediated platelet activation, suggesting the potential use of this peptide of platelet activation in therapy.

3.3 | Peptides from plants and plant-based dietary sources

Traditionally plant extracts have been used to treat blood coagulation–related ailments.[83] The cyclic depsipeptide, FR900359, isolated from the leaves of *Ardisia crenata* Sims (coralberry, from the family Primulaceae) has demonstrated marked inhibition of platelets aggregation in rabbits, decreasing blood pressure and leading to hypotension in anesthetized normotensive rats.[84] Using bioassay-guided fractionation, two novel derivative peptides, fluorine moiety-L-Val-L-Phe-OMe (ixorapeptide I) and L-Ile-N, N-dimethyl Phe (ixorapeptide II), have been sourced from the methanolic extract of *Ixora coccinea* (flame of the woods from the family Rubiaceae). In an assay ixorapeptide I has exhibited antiplatelet activity with an IC_{50} of 29.52 μg/mL.[85] *Bauninia forficata* leave-derived cysteine proteinase baupain hindered thrombin-induced platelet aggregation.

Proteins from dietary plants such as oats (*Avena sativa*), highland barley (*Hordeum vulgare*), and buckwheat (*Fagopyrum esculentum*) were enzymatically digested by gastrointestinal trypsin and alcalase, to release peptides. In in vitro assays, these hydrolysates have demonstrated high antiplatelet potential in a dose-dependent manner with IC_{50} values varying from 0.282 mg/m (oat flour gastrointestinal hydrolysate, 6 hours) to 2.496 mg/mL (highland barley glutelin tryptic hydrolysate, 14 hours). The findings suggest that the modification of grain flour by means of a proteases may produce a beneficial outcome on platelets aggregation.[77,86–88] In another study, peptides released following trypsin digestion of oats (globulins, glutelins), highland barley (albumins, glutelins), and buckwheat (albumins, glutelins) proteins were tested for antiplatelet activity.[89,90] The results showed that peptides from buckwheat and oat inhibited platelets aggregation in a dose-dependent fashion following 14 hours of hydrolysis, with a 60% effect at 0.5 mg/mL, whereas the protein fractions from highland barley did not show any anti-aggregation activity. Tryptic hydrolysate derived from oat globulin exhibited the highest potency giving an IC_{50} value of 0.307 mg/mL following 14 hours enzymatic incubation. These findings suggest additional antiplatelet peptides may be present in the tryptic hydrolysates from oat and buckwheat proteins. Proteomic analysis of oat globulin tryptic hydrolysate revealed 38 individual peptides. Most of these were long peptides consisting of over seven amino acid residues.[77,91] Soybean acid peptides (glutamate-glutamate and aspartate-aspartate-aspartate) along with the isoflavone genistein were found to reduce the activation of platelets by collagen and ADP, which might have a protective effect against coronary atherosclerosis.[92] Bromelain and papain prevent human platelets aggregation, by the unspecific cleavage in the Phe-Leu bond of protease-activated receptor 1 (PAR1). A mouse model study showed that grape seed extracts inhibited platelet aggregation in a dose-dependent manner by inhibiting tyrosine phosphatase activity.

3.4 | Peptides from animal-based dietary sources

Milk proteins have been found to generate a myriad of bioactive peptides, including antithrombotic peptides which can inhibit fibrinogen binding to platelet surfaces.[93]

Sheep casein consists of a C-part terminal referred to as caseinoglycopeptide, which inhibited the aggregation of platelets induced by collagen and thrombin, based on the doses used. The peptides RGDF and KDQDK present in the protein were held responsible for the prevention of aggregation. Elastin peptides from fish and bovine sources have also been found to reduce the aggregation of platelets induced by collagen.[94]

3.5 | Peptides from other natural sources

A murine monoclonal antibody, AC7 (IgM), has been produced against a synthetic peptide located within the RGD-binding region on GPIIIa subunit (residues 109-128) and was shown to interact only with activated platelets. The AC7-activated platelets interaction was inhibited by fibrinogen and RGD (Arg-Gly-Asp)-containing peptides. Furthermore, AC7 has been inhibited fibrinogen binding and platelet aggregation in a dose-dependent fashion. To identify the regions of AC7 that interact with the receptor to inhibit platelet GP IIb/IIIa functions the decapeptide RQMIRGYFDV (H3) was synthesized and tested for its platelet aggregation inhibition potential and binding of fibrinogen to platelets stimulated by ADP.[73,95] Among the six complementarity-determining regions (CDRs) of AC7, the CDR3 heavy chain was found to be homologous to the RGDF sequence in fibrinogen (Aα chain). The synthetic peptide encircling the RQMIRGYFDV region was found to inhibit aggregation of platelets and prevent binding of fibrinogen at a concentration of 700 μm (IC_{50}).[73] However, this peptide was less potent as compared to the antibody itself which has an IC_{50} of 105 nM.

In contrast, a plethora of GP IIb/IIIa antagonists such as abciximab, eptifibatide, and tirofiban are widely used in the clinic. Through time these pharmacological agents have demonstrated distinguished safety and efficacy profiles notably in coronary arterial interventions. A study assayed the inhibitory potential of abciximab, tirofiban, and

eptifibatide on shear-induced platelet aggregation and adhesion. The IC_{50} values recorded for the three GP IIb/IIIa antagonists were 43, 430, and 5781 nM, respectively.[95]

In the saliva of *Lutzomyia ayacuchensis* (a sand fly species and the vector of *Leishmania mexicana* and *Leishmania peruviana)* , a novel peptide ayadualin with an RGD (Arg-Gly-Asp) was found. In its mature form, ayadualin includes 20 amino acids, and it inhibits the aggregation of platelets induced by either collagen or ADP, in a dose-dependent manner with IC_{50} values of 8.37 and 5.66 μM, respectively.[96] The substitution of cysteine residues flanking the RGD sequence to serine residues (CS mutant) revoked its inhibition on platelets, stalwartly advocating the significance of the disulfide bond located on both sides of ayadualin in exerting its antiplatelet activity. Tick salivary gland mature peptides of 39 to 47 amino acid length, containing Pro/Glu(P/E)-Pro/His(P/H)-Lys-Gly-Asp (RGD) domain are disintegrins, with the ability to inhibit platelet aggregation. YY-39, one of such tick peptide blocked platelet adhesion to soluble collagen in rodent model.[74]

Many bioactive peptides have been reported to occur in the skin of amphibians but no antiplatelet peptide had been published before the Zongdian platelet inhibitor (ZDPI, 1798.6 Da), which was purified and characterized from the skin secretions of the frog *Amolops loloensis*. This peptide is composed of 15 amino acids with two cysteines that provided an intramolecular disulfide bridge and C-terminal amidation. The amino acids of ZDPI is FRGCWLKNYSPRGCL-NH2. The platelet inhibitory effects of ZDPI were tested on platelet aggregation induced by ADP and the inhibition of aggregation was found to be 36% for a concentration of 8 μg/mL, 57% for 16 μg/mL, and 89% at 32 μg/mL, tested over the course of 300 seconds. The calculated IC_{50} value was 12.27 μg/mL. ZDPI was found to inhibit platelets aggregation induced by ADP in a dose-dependent manner, with highest inhibitory effect observed at the concentrations of 32 μg/mL.[75] Its strong platelet inhibitory potential in combination with its simple structure, make it a promising candidate for designing antithrombosis drugs.

The Arg-Gly-Asp or RGD motif has been identified in high-molecular weight cell-adhesion protein in the mushroom *Lentinus edodes*. Such peptides might be explored for their potential in preventing platelet aggregation.

3.6 | Synthetic peptides

Eptifibatide (Integrilin), a cyclic heptapeptide, is a short-acting and reversible inhibitor of platelet aggregation. This peptide is based on KGD (lysine-glycine-aspartic acid) sequence similar to that found in the snake venom barbourin. This sequence is purported to antagonize the platelet GP IIb/IIIa receptor with high affinity. The potential benefits of this drug include its ability to bind reversibly which is advantageous for treating patients at high risk for bleeding.[97,98,100] A novel peptide Pro-Ser-Nva-Gly-Asp-Trp (Z4A5) was found to inhibit platelet aggregation and the formation of platelet thrombi. The activity of Z4A5 on fibrinogen and PAC-1 (an IgM monoclonal antibody) binding to GP IIb/IIIa was studied. The results indicated that Z4A5 is a potent inhibitor of human platelet aggregation in dose-dependant manner.[57,97,98] A type III collagen-derived octapeptide (Lys-Pro-Gly-Glu-Pro-Gly-Pro-Lys) has demonstrated marked inhibitory activity against aggregation of platelets. The inhibitory effect of the octapeptide varied from 34.5% (0.25 mM) to 100% (2 mM).[77,85]

One of the critical issues which need to be considered while developing peptide-based drugs is their low potency. A study revealed that protein-protein interaction sites flanked by proline (Pro) residues promote their interactions. Protein domains, including SH3, WW, and EVH1 have motifs for proline recognition in polyproline ligands,[86] mediating signaling. An antiplatelet peptide, IARGDMNA was selected to test this theory, with its RGD tripeptide as a potential target site.[87,88] In small peptides, the RGD motif prevents platelet aggregation. The insertion of a single proline residue, whether on the amino or the carboxyl side of the site of interaction, enhanced the antiplatelet effect between 1.5 and 2.5 times, while the insertion onto both sides was found to increase activity between 7 and 13 times. The peptide with complete proline brackets (IPRGDMPA), was found to inhibit platelet aggregation with an IC_{50} of 2.2 to 4.0 μM. This implies that the antiplatelet action of the peptide may have been augmented because of a lower number of conformational possibilities for the peptide yet avoiding the rigidity which would come with cyclization.[88,89]

4 | DISCUSSION

The majority of the peptide sequences mentioned here are either from natural sources or are derived forms, ranging from 3 to 15 amino acids. Glu-Asn-Trp was the smallest peptide found to have antiplatelet action with low risk of allergic reactions. Lebetin peptides have proved to be the most potent of the tested peptides. However, due to their long sequences, they require strategies to preserve their pharmacological potency after administration.

Peptides with up to five amino acids may be administered through the oral route, without any pharmaceutical modification. Their plasma half-lives and bioavailability is good as they can resist the host proteases. However, peptides with more than five amino acids are prone to hydrolysis in the harsh gastrointestinal environment and may need parenteral administration or stealthy pharmaceutical dosage forms. These approaches for better oral delivery, and stability include proteinylation, PEGylation, glycosylation, metabolism modification, and so forth.

While the antiplatelet aggregation activity of peptides appears unusual when considering their therapeutic importance, there are several caveats which must be considered while drawing inferences. The human body elaborates several stress peptides such as corticotropin-releasing hormone (CRH) which mediate the production of inflammatory cytokines (TNF-α and interleukin-6) and adhesion molecules.[90] Platelet and endothelial cell adhesion molecule 1 are found on the surface of platelets. The deposition of amyloid β-peptide (Aβ), a proteolytic fragment of the amyloid precursor protein (APP), in senile plaques and in the brain tissue of patients is the hallmark of Alzheimer's disease (AD). Platelets contain both APP and Aβ, and may contribute to the perivascular amyloid deposition seen in AD. However, the precise mechanisms(s) involved in their formation and release by these cells have not been identified.[91] It has been documented that human platelets release Aβ and APP, when activated by a wide range of agonists such as thrombin or collagen. While this mechanism is still unclear, several studies have highlighted on the link between the plaque-forming peptides and platelets.[91–94]

Bacterial collagenase degrades endothelial extracellular matrix, releasing proangiogenic short peptides. Certain peptides produced by plants, such as hevein, contain chitin-binding domains,[73] which can be allergenic to humans. The peptide gliadin, derived from gluten, is responsible for the inflammation of gut mucosa and Celiac disease.[95,96] Allergens are perceived as stressors, which result in the activation of the human immune system. Subsequent blood coagulation is part of this defense mechanism.

In addition to their platelet inhibitory effects, snake venom-derived peptides acting as anticoagulants is intriguing, as depending on the dosage of the venom, its proteinases may instead act as procoagulants. Convulxin, a protein in the venom of *Crotalus durissus terrificus* (a rattlesnake) is responsible for aggregating platelets. This protein binds to the collagen receptor in platelets, glycoprotein VI.[74] In fact, the major component of snake venom is metalloprotease, which can be lethal to the snake itself. So, antagonists occur in the venom. Metalloprotease such as halysase have the disintegrin-like domains, which can inhibit human platelet aggregation.

Blood coagulation is a double-edged sword. It is a defense strategy, so the attempt to meddle with this process can be dangerous. Also, the reports of peptides preventing platelets aggregation can be the outcome of bad experimental design or reflective of an incomplete picture. The structural modifications of the peptides can alter their platelet inhibition potential. It indicates the extreme relevance of charge in the platelet aggregation. In this regard, it can be explored, if the pH of the host milieu determines the efficacy of peptides. Also, the fate of the peptides in the host body should be monitored, as it might be metabolized before exerting the inhibitory action.

Proteins, and their building blocks amino acids, can act as signaling molecules. Peptides are entities occurring in between these, with an understandable role in signaling themselves. Peptides with a repeated Gly-Pro-Hyp sequence are platelet agonists, as this motif is recognized by platelet glycoprotein VI.[75] Immunogenicity of peptides must be considered while contemplating their use in manipulating platelets. Like drugs, their relevance might be dependent on the age, gender, and comorbidity of a patient. If inflammatory milieu prevails in the individuals, peptides may not inhibit platelets as assumed.

As mentioned before, blood circulation in normal physiological conditions, and coagulation while encountering stressors, is the evolution-designed fundamental criteria on which survival depends. So, employing peptides for the modulation of blood flow properties, by the manipulation of cognate receptors and enzymes can be risky. Platelet-derived microparticles have an abundance of proteins, including GP IIIa, GP IIb, P-selectin, and chemokines (CXCL4, CXCL7, and CCL5). Thus, the interaction between these proteins and any potential antiplatelet peptide ought to be studied. When activated under inflammatory milieu, the platelets themselves elaborate a range of peptides, with platelet factor 4, connective tissue activating peptide 3, platelet necessary protein, normal T cell expressed and secreted, thymosin beta-4, fibrinopeptide B, and fibrinopeptide A, as examples.[99] Targeting a component which endogenously releases peptides is therefore, technically difficult. Hence, it is critical to investigate the candidate peptides, to ascertain their safety.

5 | CONCLUSION

Platelet-dependent thrombus formation is a key event in the pathogenesis of coronary atherothrombotic diseases, and antiplatelet therapy is the cornerstone for managing thrombotic diseases. As current drug arsenal to resolve platelet aggregation is limited, this review highlights the natural and synthetic peptides with noticeable antiplatelet activity. Peptides and proteins of natural origin, derived from sources such as snake venom, or dietary plants, among others, have demonstrated significant antiplatelet activity. The plausible mechanisms of antiplatelet action for these peptides and proteins have been discussed. Future development of new antiplatelet and thrombolytic agents will be compelled to contend with two major constraints. First, for many clinical indications in this field, highly effective, and inexpensive antiplatelet drugs such as aspirin and clopidogrel are already available. Second, antithrombotic and thrombolytic agents are associated with bleeding issues, which are likely to hinder new agents under development. Nonetheless, scope remains for the development of more effective and safer therapeutic agents. Likewise, the 20-residue synthetic peptide bivalirudin is a genuine goal of such aim. It is clinically used in non-ST segment elevated myocardial infarction and left ventricular assist device thrombosis, inhibiting both thrombin and platelet adhesion. When compared to heparin, it is less immunogenic and is associated with lower risks of major bleeding. In conclusion, more carefully-designed in vitro, in vivo and clinical antiplatelet studies are recommended to accelerate drug development in this field.

ACKNOWLEDGEMENTS

The author KRRR thanks the DST-SERB, New Delhi for financial support in the form of postdoctoral fellowship (File. No. PDF/2017/001166/LS). The authors KRRR and SKP sincerely acknowledge the computational and bioinformatics facility provided by the Alagappa University Bioinformatics Infrastructure Facility (funded by DBT, GOI; File No. BT/BI/25/012/2012, BIF). The author SKP also thank RUSA 2.0 (F. 24 to 51/2014-U, Policy [TN Multi-Gen], Department of Education, Government of India). SX is a recipient of Career Development Award (#18CDA34110359) from American Heart Association.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

1. McNicol A, Israels SJ. Beyond hemostasis: the role of platelets in inflammation, malignancy and infection. *Cardiovascular & Haematological Disorders-Drug Targets (Formerly Current Drug Targets-Cardiovascular & Hematological Disorders)*. 2008;8(2):99-117.
2. Bergmeier W, Hynes RO. Extracellular matrix proteins in hemostasis and thrombosis. *Cold Spring Harbor Perspectives in Biology*. 2012;4(2):a005132.
3. Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arteriosclerosis, thrombosis, and vascular biology*. 2008;28(3):403-412.
4. Lei H, Kazlauskas A. Growth factors outside of the platelet-derived growth factor (PDGF) family employ reactive oxygen species/Src family kinases to activate PDGF receptor alpha and thereby promote proliferation and survival of cells. *The Journal of biological chemistry*. 2009;284(10):6329-6336.
5. Gale AJ. Continuing Education Course #2: Current Understanding of Hemostasis (2011;39(1):273-280.
6. Whiteheart SW. Platelet granules: surprise packages. *Blood*. 2011;118(5):1190-1191.
7. Golebiewska EM, Poole AW. Platelet secretion: From haemostasis to wound healing and beyond. *Blood reviews*. 2015;29(3):153-162.
8. Armstrong AW, Golan DE. Pharmacology of hemostasis and thrombosis. *Principles of Pharmacology: The Pathophysiologic Basis of Drug Therapy*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins. 2011:372-400.
9. Dovizio M, Alberti S, Guillem-Llobat P, Patrignani P. Role of platelets in inflammation and cancer: novel therapeutic strategies. *Basic & Clinical Pharmacology & Toxicology*. 2014;114(1):118-127.
10. Ghoshal K, Bhattacharyya M. Overview of platelet physiology: its hemostatic and nonhemostatic role in disease pathogenesis. *The Scientific World Journal*. 2014:2014.
11. Shin E-K, Park H, Noh J-Y, Lim K-M, Chung J-H. Platelet shape changes and cytoskeleton dynamics as novel therapeutic targets for anti-thrombotic drugs. *Biomolecules & Therapeutics*. 2017;25(3):223.
12. Lyden D, Hattori K, Dias S, et al. Impaired recruitment of bone-marrow–derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nature Medicine*. 2001;7(11):1194-1201.
13. Nesbitt WS, Westein E, Tovar-Lopez FJ, et al. A shear gradient–dependent platelet aggregation mechanism drives thrombus formation. *Nature medicine*. 2009;15(6):665.
14. Kaplan ZS, Jackson SP. The role of platelets in atherothrombosis. *ASH Education Program Book*. 2011;2011(1):51-61.
15. Steg PG, Dorman S, Amarenco P. Atherothrombosis and the role of antiplatelet therapy. *Journal of Thrombosis and Haemostasis*. 2011;9:325-332.
16. Meade T. Primary prevention of ischaemic cardiovascular disorders with antiplatelet agents. *Antiplatelet Agents. Springer*. 2012:565-605.
17. Banerjee S, Angiolillo DJ, Boden WE, et al. Use of antiplatelet therapy/DAPT for post-PCI patients undergoing noncardiac surgery. *Journal of the American College of Cardiology*. 2017;69(14):1861-1870.
18. Davì G, Patrono C. Platelet activation and atherothrombosis. *New England Journal of Medicine*. 2007;357(24):2482-2494.
19. Fintel DJ. Oral antiplatelet therapy for atherothrombotic disease: overview of current and emerging treatment options. *Vascular Health and Risk Management*. 2012;8:77.
20. Friedman EA, Ogletree ML, Haddad EV, Boutaud O. Understanding the role of prostaglandin E2 in regulating human platelet activity in health and disease. *Thrombosis research*. 2015;136(3):493-503.
21. Yun S-H, Sim E-H, Goh R-Y, Park J-I, Han J-Y. Platelet Activation: The Mechanisms and Potential Biomarkers %J BioMed Research International. 2016;2016(5).
22. Offermanns S. Activation of platelet function through G protein–coupled receptors. *Circulation Research*. 2006;99(12):1293-1304.
23. Meadows TA, Bhatt DL. Clinical aspects of platelet inhibitors and thrombus formation. *Circulation Research*. 2007;100(9):1261-1275.
24. Fanaroff AC, Rao SV. Antiplatelet Therapy in Percutaneous Coronary Intervention. *Interventional cardiology clinics*. 2016;5(2):221-237.

25. Ferraris VA, Ferraris SP, Saha SP. Antiplatelet drugs: mechanisms and risks of bleeding following cardiac operations. *The International Journal of Angiology: official publication of the International College of Angiology, Inc.* 2011;20(1):1.
26. Angiolillo DJ, Guzman LA, Bass TA. Current antiplatelet therapies: benefits and limitations. *American Heart Journal*. 2008;156(2):3S-9S.
27. Bliden KP, DiChiara J, Tantry US, Bassi AK, Chaganti SK, Gurbel PA. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *Journal of the American College of Cardiology*. 2007;49(6):657-666.
28. Varon D, Spectre G. Antiplatelet agents. *ASH Education Program Book*. 2009;2009(1):267-272.
29. Hawkey C. Cyclooxygenase inhibition: between the devil and the deep blue sea. *Gut*. 2002;50(suppl 3):iii25-iii30.
30. Gresele P. Antiplatelet agents in clinical practice and their haemorrhagic risk. *Blood Transfusion*. 2013;11(3):349.
31. Floyd CN, Ferro A. Antiplatelet drug resistance: Molecular insights and clinical implications. *Prostaglandins and Other Lipid Mediators*. 2015;120:21-27.
32. Homma S, Thompson JLP, Pullicino PM, et al. Warfarin and Aspirin in Patients with Heart Failure and Sinus Rhythm. *New England Journal of Medicine*. 2012;366(20):1859-1869.
33. Patrono C, Rocca B. Aspirin: promise and resistance in the new millennium. *Arteriosclerosis, thrombosis, and vascular biology*. 2008;28(3):s25-s32.
34. Meher S, Duley L, Hunter K, Askie L. Antiplatelet therapy before or after 16 weeks' gestation for preventing preeclampsia: an individual participant data meta-analysis. *American Journal of Obstetrics and Gynecology*. 2017;216(2):121-128. e122.
35. Meyer EA, Caroff E, Riederer MA. Advances in Antiplatelet Agents. *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*. Elsevier; 2016.
36. Stegnar M. Platelet function tests and resistance to antiplatelet therapy. *Srpski arhiv za celokupno lekarstvo*. 2010;138(suppl. 1):59-63.
37. Capodanno D, Angiolillo DJ. Antiplatelet Therapy After Implantation of Bioresorbable Vascular Scaffolds: A Review of the Published Data, Practical Recommendations, and Future Directions. *JACC: Cardiovascular Interventions*. 2017;10(5):425-437.
38. Ueshima D, Ashikaga T, Yoshikawa S, et al. Effect of over-2-year dual antiplatelet therapy on the rate of major adverse cardiac and cerebral events for everolimus-eluting stent implantation: The landmark analysis from Tokyo-MD PCI registry. *Journal of Cardiology*. 2017;69(6):815-822.
39. Ashok NR, Aparna HS. Empirical and bioinformatic characterization of buffalo (*Bubalus bubalis*) colostrum whey peptides & their angiotensin I-converting enzyme inhibition. *Food Chemistry*. 2017;228:582-594.
40. Lu L, Qi H, Zhu J, et al. Vascular-homing peptides for cancer therapy. *Biomedicine and Pharmacotherapy*. 2017;92:187-195.
41. Luz C, Saladino F, Luciano FB, Mañes J, Meca G. In vitro antifungal activity of bioactive peptides produced by Lactobacillus plantarum against Aspergillus parasiticus and Penicillium expansum. *LWT - Food Science and Technology*. 2017;81:128-135.
42. Ortiz-Martinez M, Gonzalez de Mejia E, García-Lara S, Aguilar O, Lopez-Castillo LM, Otero-Pappatheodorou JT. Antiproliferative effect of peptide fractions isolated from a quality protein maize, a white hybrid maize, and their derived peptides on hepatocarcinoma human HepG2 cells. *Journal of Functional Foods*. 2017;34:36-48.
43. Ruiz-Santaquiteria M, Sánchez-Murcia PA, Toro MA, et al. First example of peptides targeting the dimer interface of Leishmania infantum trypanothione reductase with potent in vitro antileishmanial activity#. *European Journal of Medicinal Chemistry*. 2017;135:49-59.
44. Santhekadur PK, Kumar DP, Seneshaw M, Mirshahi F, Sanyal AJ. The multifaceted role of natriuretic peptides in metabolic syndrome. *Biomedicine and Pharmacotherapy*. 2017;92:826-835.
45. Shazly AB, He Z, El-Aziz MA, et al. Fractionation and identification of novel antioxidant peptides from buffalo and bovine casein hydrolysates. *Food Chemistry*. 2017;232:753-762.
46. Wang X, Junior JCB, Mishra B, Lushnikova T, Epand RM, Wang G. Arginine-lysine positional swap of the LL-37 peptides reveals evolutional advantages of the native sequence and leads to bacterial probes. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2017;1859(8):1350-1361.
47. Padhi A, Sengupta M, Sengupta S, Roehm KH, Sonawane A. Antimicrobial peptides and proteins in mycobacterial therapy: current status and future prospects. *Tuberculosis*. 2014;94(4):363-373.
48. Robinson SD, Safavi-Hemami H, McIntosh LD, Purcell AW, Norton RS, Papenfuss AT. Diversity of conotoxin gene superfamilies in the venomous snail, Conus victoriae. *PLoS One*. 2014;9(2):e87648.
49. Benimana O, Zhao L, Kong Y, Li Z, Xie Z. The progress in the research of antiplatelet agents (1995–2017). *Future Medicinal Chemistry*. 2017;9(10):1087-1110.
50. Sun L. Peptide-based drug development. *Modern Chemistry and Applications*. 2013;1(1):1-2.
51. Scognamiglio L, Di Natale P, Perretta C, Marasco G, From D. peptides to small molecules: an intriguing but intricated way to new drugs. *Current Medicinal Chemistry*. 2013;20(31):3803-3817.

52. Kaspar AA, Reichert JM. Future directions for peptide therapeutics development. *Drug discovery today*. 2013;18(17-18): 807-817.
53. Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. *Drug discovery today*. 2015;20(1):122-128.
54. Udenigwe CC, Fogliano V. Food matrix interaction and bioavailability of bioactive peptides: Two faces of the same coin? *Journal of Functional Foods*. 2017;35:9-12.
55. Gremmel T, Xhelili E, Steiner S, Koppensteiner R, Kopp CW, Panzer S. Response to antiplatelet therapy and platelet reactivity to thrombin receptor activating peptide-6 in cardiovascular interventions: Differences between peripheral and coronary angioplasty. *Atherosclerosis*. 2014;232(1):119-124.
56. Ding B, Xu Z, Qian C, et al. Antiplatelet aggregation and antithrombosis efficiency of peptides in the snake venom of Deinagkistrodon acutus: Isolation, identification, and evaluation. *Evidence-Based Complementary and Alternative Medicine*. 2015:2015.
57. Chen M, Ye X, Ming X, et al. A novel direct factor Xa inhibitory peptide with anti-platelet aggregation activity from Agkistrodon acutus venom hydrolysates. *Scientific Reports*. 2015:5.
58. Di L. Strategic approaches to optimizing peptide ADME properties. *The AAPS journal*. 2015;17(1):134-143.
59. Pawar VK, Meher JG, Singh Y, Chaurasia M, Reddy BS, Chourasia MK. Targeting of gastrointestinal tract for amended delivery of protein/peptide therapeutics: strategies and industrial perspectives. *Journal of Controlled Release*. 2014;196:168-183.
60. Miner-Williams WM, Stevens BR, Moughan PJ. Are intact peptides absorbed from the healthy gut in the adult human? *Nutrition research reviews*. 2014;27(2):308-329.
61. Houston ME, Campbell AP, Lix B, Kay CM, Sykes BD, Hodges RS. Lactam bridge stabilization of α-helices: the role of hydrophobicity in controlling dimeric versus monomeric α-helices. *Biochemistry*. 1996;35(31):10041-10050.
62. Ferdinandi ES, Brazeau P, High K, Procter B, Fennell S, Dubreuil P. Non-Clinical Pharmacology and Safety Evaluation of TH9507, a Human Growth Hormone-Releasing Factor Analogue. *Basic & Clinical Pharmacology & Toxicology*. 2007;100(1):49-58.
63. Grigoryev Y. Stapled peptide to enter human testing, but affinity questions remain. Nature Publishing Group; 2013.
64. Knudsen L. Liraglutide: the therapeutic promise from animal models. *International Journal of Clinical Practice*. 2010;64(s167):4-11.
65. Bao W, Holt LJ, Prince RD, et al. Novel fusion of GLP-1 with a domain antibody to serum albumin prolongs protection against myocardial ischemia/reperfusion injury in the rat. *Cardiovascular Diabetology*. 2013;12(1):148.
66. Kim D-S, Ji HD, Rhee MH, et al. Antiplatelet activity of Morus alba leaves extract, mediated via inhibiting granule secretion and blocking the phosphorylation of extracellular-signal-regulated kinase and akt. *Evidence-Based Complementary and Alternative Medicine*. 2014;2014. https://doi.org/10.1155/2014/639548
67. Fan Z, Zhou L, Xiong T, et al. Antiplatelet aggregation triterpene saponins from the barks of Ilex rotunda. *Fitoterapia*. 2015;101:19-26.
68. El Haouari M, Rosado JA. Medicinal Plants with Antiplatelet Activity. *Phytotherapy Research*. 2016;30(7):1059-1071.
69. Faggioni M, Baber U, Sartori S, et al. Incidence, Patterns, and Associations Between Dual-Antiplatelet Therapy Cessation and Risk for Adverse Events Among Patients With and Without Diabetes Mellitus Receiving Drug-Eluting Stents: Results From the PARIS Registry. *JACC: Cardiovascular Interventions*. 2017;10(7):645-654.
70. Lee W, Lee J, Kulkarni R, et al. Antithrombotic and antiplatelet activities of small-molecule alkaloids from *Scolopendra subspinipes* mutilans. *Scientific Reports*. 2016;6. https://doi.org/10.1038/srep21956
71. Metcalfe C, Ramasubramoni A, Pula G, Harper MT, Mundell SJ, Coxon CH. Thioredoxin inhibitors attenuate platelet function and thrombus formation. *PLoS ONE*. 2016;11(10):e0163006.
72. Hasan AA, Amenta S, Schmaier AH. Bradykinin and its metabolite, Arg-Pro-Pro-Gly-Phe, are selective inhibitors of alpha-thrombin-induced platelet activation. *Circulation*. 1996;94(3):517-528.
73. Murphey LJ, Malave HA, Petro J, et al. Bradykinin and its metabolite bradykinin 1-5 inhibit thrombin-induced platelet aggregation in humans. *The Journal of pharmacology and experimental therapeutics*. 2006;318(3):1287-1292.
74. Clapp C, Thebault S, Jeziorski MC. Escalera GMDL. Peptide Hormone Regulation of Angiogenesis 2009;89(4): 1177-1215.
75. Castan-Laurell I, Masri B, Valet P. The apelin/APJ system as a therapeutic target in metabolic diseases. *Expert opinion on therapeutic targets*. 2019;23(3):215-225.
76. Peltonen T, Napankangas J, Vuolteenaho O, et al. Apelin and its receptor APJ in human aortic valve stenosis. *The Journal of heart valve disease*. 2009;18(6):644-652.
77. Pitkin SL, Maguire JJ, Kuc RE, Davenport AP. Modulation of the apelin/APJ system in heart failure and atherosclerosis in man. *British journal of pharmacology*. 2010;160(7):1785-1795.
78. Kuklinska AM, Sobkowicz B, Sawicki R, et al. Apelin: a novel marker for the patients with first ST-elevation myocardial infarction. *Heart and vessels*. 2010;25(5):363-367.
79. Li Z, Bai Y, Hu J. Reduced apelin levels in stable angina. *Internal medicine (Tokyo, Japan)*. 2008;47(22):1951-1955.

80. Adam F, Khatib AM, Lopez JJ, et al. Apelin: an antithrombotic factor that inhibits platelet function. *Blood*. 2016;127(7):908-920.
81. Lyapina L, Pastorova V, Obergan TY, Samonina G, Ashmarin I, Myasoedov N. Comparison of anticoagulant effects of regulatory proline-containing oligopeptides. *Specificity of glyprolines, semax, and selank and potential for their practical application. Biology Bulletin*. 2006;33(2):153-161.
82. Karniguian A, Legrand Y, Lefrancier P, Caen J. Effect of a collagen derived octapeptide on different steps of the platelet/collagen interaction. *Thrombosis Research*. 1983;32(6):593-604.
83. Zarrinpar A, Bhattacharyya RP, Lim WA. The structure and function of proline recognition domains. *Sci STKE*. 2003;2003(179):re8-re8.
84. Kini RM, Evans HJ. Prediction of potential protein-protein interaction sites from amino acid sequence: Identification of a fibrin polymerization site. *FEBS letters*. 1996;385(1–2):81-86.
85. Kini RM, Evans HJ. A novel approach to the design of potent bioactive peptides by incorporation of proline brackets: antiplatelet effects of Arg-Gly-Asp peptides. *FEBS Letters*. 1995;375(1-2):15-17.
86. Samuel D, Ganesh G, Yang PW, et al. Proline inhibits aggregation during protein refolding. *Protein Science*. 2000; 9(2):344-352.
87. Theoharides TC, Tsilioni I, Arbetman L, et al. Fibromyalgia syndrome in need of effective treatments. *Journal of Pharmacology and Experimental Therapeutics*. 2015;355(2):255-263.
88. Skovronsky DM, Lee VM-Y, Praticò D. Amyloid precursor protein and amyloid β peptide in human platelets Role of cyclooxygenase and protein kinase C. *Journal of Biological Chemistry*. 2001;276(20):17036-17043.
89. Canobbio I, Abubaker AA, Visconte C, Torti M, Pula G. Role of amyloid peptides in vascular dysfunction and platelet dysregulation in Alzheimer's disease. *Frontiers in Cellular Neuroscience*. 2015;9:65.
90. Li G-X, Whyte S, Tanner JE, Eyin G, Beyreuther K. Secretion of Alzheimer's Disease Aß Amyloid Peptide. *Laboratory Investigation*. 1998;78(4):461.
91. Inyushin MY, Sanabria P, Rojas L, Kucheryavykh Y, Kucheryavykh L. Aβ Peptide Originated from Platelets Promises New Strategy in Anti-Alzheimer's Drug Development. *BioMed Research International*. 2017:2017.
92. Kini SG, Nguyen PQ, Weissbach S, et al. Studies on the chitin binding property of novel cysteine-rich peptides from alternanthera sessilis. *Biochemistry*. 2015;54(43):6639-6649.
93. Bernardo D, Garrote J, Fernández-Salazar L, Riestra S, Arranz E. Is gliadin really safe for non-coeliac individuals? Production of interleukin 15 in biopsy culture from non-coeliac individuals challenged with gliadin peptides. *Gut*. 2007;56(6):889-890.
94. Hollon J, Puppa EL, Greenwald B, Goldberg E, Guerrerio A, Fasano A. Effect of gliadin on permeability of intestinal biopsy explants from celiac disease patients and patients with non-celiac gluten sensitivity. *Nutrients*. 2015;7(3): 1565-1576.
95. Niedergang F, Alcover A, Knight C, et al. Convulxin binding to platelet receptor GPVI: competition with collagen related peptides. *Biochemical and biophysical research communications*. 2000;273(1):246-250.
96. Knight CG, Morton LF, Onley DJ, et al. Collagen–platelet interaction: Gly-Pro-Hyp is uniquely specific for platelet Gp VI and mediates platelet activation by collagen. *Cardiovascular research*. 1999;41(2):450-457.
97. Tang Y-Q, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. *Infection and immunity*. 2002;70(12):6524-6533.
98. Plow EF, Marguerie G Inhibition of fibrinogen binding to human platelets by the tetrapeptide glycyl-L-prolyl-L-arginyl-L-proline. *Proceedings of the National Academy of Sciences*. 1982;79(12):3711-3715.
99. Legrand YJ, Karniguian A, Le Francier P, Fauvel F, Caen JP. Evidence that a collagen-derived nonapeptide is a specific inhibitor of platelet-collagen interaction. *Biochemical and Biophysical Research Communications*. 1980;96(4): 1579-1585.
100. Li Y-X, Sun Q, Zhang H, et al. A novel anti-platelet peptide (Z4A5) potential for glycoprotein IIb/IIIa inhibits platelet aggregation. *Thrombosis Research*. 2012;129(5):e217-e222.

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Foundation fellowship and National Research Foundation—South Africa Innovation postdoctoral fellowship. He is a member of Society for Medicinal Plant and Natural Product Research. He is Senior Editor of Plant Physiology and Molecular Plant Pathology, Associate Editor of Frontier's in Marine Science, Frontier's in Nutrition and editorial board member of Marine Drugs, South African Journal of Botany, Pharmacological Research, Phytomedicine, and Frontier's in Pharmacology.

Haroon Khan is a graduate of University of Peshawar, Pakistan. Dr Khan has started his professional carrier in 1999. From lecturer, selected as Associate Professor and Principal Gandhara University, Peshawar. In Abdul Khan University Mardan, he has started the Department of Pharmacy as a Chairman in 2013 and now holding the status of a full professor and chairman of the department. Research area of Dr Khan is natural product isolation and pharmacological activities. In this regard, he has published more than 190 articles and written several books/book chapters. His research contribution is acknowledged by PCST and granted research productivity award five times. He is also the editorial board member of several international journals.

Imad Ahmad is from Mardan, Pakistan. He received his Pharm-D degree from Hazara University Mansehra and MPhil (Pharmaceutical sciences) from Abdul Wali Khan University Mardan, Pakistan. His MPhil Research was based on drug discovery through enzyme inhibition and molecular modeling. Currently, he is involved in teaching and research at Pharmacy Department of Abasyn University Peshawar, Pakistan. His research interests lie in drug discovery, Pharmacology and Pharmacy practice. He also has an experience in Pharmaceutical formulations.

Devina Lobine holds in degree Agricultural Biotechnology, following she has read her MPhil/PhD degree (Natural Product) in close collaboration between the University of Mauritius, Durham University (UK) and University of Pretoria (South Africa). She is currently registered as post-doctoral fellow at the University of Mauritius, investigating "Herbal phosphodiesterase inhibitors as therapeutics for managing Alzheimer's disease." Devina has authored/coauthored 19 publications (11 published scientific papers; four book sections and four published abstracts). In her role as the Youth Ambassador for Southern Africa Network for Biosciences, she is dedicating uncountable time in promoting life sciences through activities, not only in Mauritius but also across the Southern African region.

Shanoo Suroowan is currently a PhD student at the University of Mauritius. His research focuses on the elucidation of the pharmacological properties of poorly studied yet commonly used plant species by the Mauritian population as well as assessing their herb-drug interaction potential. Mr Suroowan obtained his Bachelor of Pharmacy degree in 2014. Succeeding the accomplishment of his pharmacy degree, he has had more than 1 year experience as pharmacist in charge in various private pharmacies in Mauritius. He is also member of the international advisory board for the global alliance for infections in surgery and the young scholar global committee. Though his career as a researcher is still in its infancy phase Mr Suroowan has authored or coauthored over more than 10 research papers, review articles, and book chapters.

Fawzi Mahomoodally holds a life sciences degree and a PhD in Biochemistry. He is a Harvard University Alumni and has worked as a clinical trial manager for a contract research organization involved in clinical studies. He is presently a full time academic and was promoted on fast track from lecturer to senior lecturer and then to associate professor at the Department of Health Sciences, University of Mauritius. He headed the department of health sciences from 2016 to 2018. Fawzi has authored 300 scientific publications, guided more than >8 PhD students and reviewer/editor of >100 scientific journals. He is recipient of >50 fellowships/travel grants/plenary speaker to attend international seminars, teaching tool workshops/conferences. He is presently the Principal Investigator (PI) and co-PI of seven research grants/consortium regionally/internationally.

Sherif T.S. Hassan is Regents' Researcher of Pharmacognosy at Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences (UVPS), Brno, Czech Republic. Dr Hassan has obtained his PhD degree in Pharmacognosy from UVPS, Brno, Czech Republic. In addition to his role as a lecturer and researcher at UVPS, Dr Hassan is a member of editorial board of International journals such as Frontiers in Pharmacology, Frontiers in Microbiology, and Frontiers in nutrition. He is an active member of several international societies such as Czech Pharmaceutical Society, the American Society of Pharmacognosy, Phytochemical Society of Europe, American Society for Pharmacology and Experimental Therapeutics, and International Union of Basic and Clinical Pharmacology.

Seema Patel is a Graduate of Indian Institute of Technology Guwahati, India and San Diego State University, United States. She has worked in industrial microbiology and then on in silico clinical microbiology. She has served as Assistant Professor in Lovely Professional University, India; as Research Assistant in San Diego State University; and Laboratory technologist in a genetic facility in California. She is involved in biomedical research, since 2007, and has written or edited 10 books, and published more than 100 papers on microbiology, food science, bioinformatics, immunology, endocrinology, and allied topics.

Suowen Xu received his PhD degree in pharmacology from Sun Yat-Sen University (Guangzhou, China) in 2011. After graduation, he did his postdoctoral research at National Heart, Lung and Blood Institute/National Institutes of Health (NHLBI/NIH, Bethesda, MD) as a visiting fellow, studying the biochemistry and function of ADP-ribosylation. In February 2014, he joined the Aab Cardiovascular Research Institute at the University of Rochester (Rochester, NY) as a postdoctoral research associate, studying epigenetic regulation of endothelial function by atheroprotective shear stress and associated vascular biology. He is currently Research Assistant Professor at University of Rochester. He has had a long-term interest in studying the molecular mechanisms of atherogenesis as well as bioactive natural products (nutraceuticals and Chinese medicine)-based drug discovery to treat atherosclerosis and related cardiometabolic diseases. He is a member of American Heart Association (AHA, since 2012) and American Association for the Advancement of Science (AAAS, since 2014). He is an editorial board member of Scientific Report, Frontiers in Pharmacology, PLOS One, BMC Complementary and Alternative Medicine, BMC Pharmacology and Toxicology, and several other journals. He is a recipient of Career Development Award (#18CDA34110359) from American Heart Association.

Syed M. Nabavi is a Biotechnologist and Senior Scientist at Baqiyatallah University of Medical Science and a member of Iran's National Elites Foundation. His research focuses on the health effects of natural products. He is the author/coauthors of 200 publications in international journals, 51 communications at national and international journals. His h-index is 33 (obtained from the Scopus database in June 2017). He is also ranked in the top 1% of scientist in the world in the field of Agricultural Sciences according to the Essential Science Indicator from Thompson Reuters ISI.

Maria Daglia obtained her degree in Chemical and Pharmaceutical Technologies at the University of Pavia in 1989 (110/110 cum laude). In 1993, she received her PhD in Chemical and Pharmaceutical Technologies, in 1996, she obtained her Specialization in "Farmacia Industriale" (50/50). She is Associate Professor in Food Chemistry at the University of Pavia. Her scientific and research activities are focused on two fields: (1) study of biological and functional properties of foods (mutagenic properties of Maillard reaction products, antioxidant and specific antiradical properties of water soluble vegetable components in chemical and biological systems, antibacterial and antiadhesive properties against plaque bacteria, antidepressive-like properties of natural compounds, either naturally present or also induced following thermo/technological treatments in foods, useful in nutraceutical, pharmaceutical and cosmetic fields and (2) development of analytical spectrophotometric and chromatographic methods (TLC, GC, GFC, HPLC, HPLC-MS) useful in the identification and determination of active compounds used in pharmaceutical industry, occurring in biological fluids and in foods. She participated to several research projects

supported by local, national and UE grants. Prof. Daglia's research activity is documented by 149 scientific papers and 120 communications to national and international congresses.

Shunmugiah K. Pandian, DSc, has been heading the Department of Biotechnology, Alagappa University, India, since March 2002. His group has been working on novel antiquorum sensing and antibiofilm agents from natural resources with special interest on marine microbial metabolites and edible natural resources and the same has been highlighted in Nature India (doi:10.1038/nindia.2012.72). He has published more than 160 research papers in SCI journals, presented 295 papers in National and International Conferences/Seminars/ Workshops, delivered 31 lectures in Refresher Courses and 46 Special/Keynote addresses and guided 20 PhD students. He is also serving as Editor for (1) Indian Journal of Microbiology (Springer), and (2) Journal of Medical Microbiology (Society for General Microbiology, UK). He has received several awards including the Tamil Nadu Scientist Award (TANSA) in Biological Sciences (2012), Shri P.K. Das Memorial Lifetime Achievement Award (2016), Malaviya Memorial Award (2017), and UGC Mid-Career Award (2017).

How to cite this article: Rengasamy KR, Khan H, Ahmad I, et al. Bioactive peptides and proteins as alternative antiplatelet drugs. *Med Res Rev*. 2019;1-19. https://doi.org/10.1002/med.21579