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Review

Immunomodulatory effects of *Sutherlandia frutescens* extracts on metabolic syndrome

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ABSTRACT

The South African medicinal plant, *Sutherlandia frutescens* (L.) R.Br. (also known as *Lessertia frutescens*) is traditionally used to treat conditions in which inflammation is a central feature. Aqueous and organic extracts of *Sutherlandia frutescens* have immune-regulatory, anti-inflammatory and antioxidant properties, potentially underlying its wide ranging medicinal activities. In this review, data relating to the effects of *Sutherlandia frutescens* extracts on the function of two major macrophage sub-populations involved in inflammation and immune responses is addressed. Findings are related to chronic inflammatory states found in obesity, type 2 diabetes and the metabolic syndrome, in which the roles of classically activated pro-inflammatory M1 macrophages and alternatively activated anti-inflammatory M2 macrophages have been highlighted. We evaluate potential mechanisms by which *Sutherlandia frutescens* extracts may regulate macrophage activation and wider metabolic immunomodulatory processes underlying these conditions and indicate potential ethnopharmacological mechanisms of action. Electronic databases from 1965 to 2019 and other literature sources, including short communications, reports, conference proceedings and theses were used to compile this review. Published work indicates that *Sutherlandia frutescens* has potential as a phytomedicine to treat inflammation and stimulate wound healing, *via* down-regulation of pro-inflammatory macrophage activity. We propose that this involves regulation of MAPK, p38 and NFκB, Akt-independent IKK and Akt-dependent GSK3β signalling pathways. Control of such signals could lead to modulation of inflammatory responses in local macrophages and regulation of macrophage apoptosis, through ERK1/2 phosphorylation. This would stimulate recruitment of macrophages in resolution of inflammation and wound repair. Differing findings for aqueous and organic extracts indicate that care is needed in sourcing plant material, choice of extract procedures, systemic or topical application and dosage used in clinical situations. Currently there is no single bioactive phytochemical identified in *Sutherlandia frutescens* that accounts for the anti-inflammatory activity. We postulate that this action is due to the combined effect of several phytochemicals.

KEYWORDS: *Sutherlandia frutescens*, inflammation, macrophage, immune response, obesity, type 2 diabetes.

1. Introduction

The South African medicinal plant, *Sutherlandia frutescens* (L.) R.Br, was originally used by the Khoi San and Nama people in extracts to wash wounds and to treat fever [1, 2]. Now it is widely used in traditional medicine to treat inflammatory conditions, cancer, diabetes, back pain, stomach pain, haemorrhoids, diarrhoea and dysentery, influenza, HIV/AIDS, tuberculosis, bronchitis, rheumatism, rheumatoid arthritis and osteo-arthritis, asthma, eye diseases and skin diseases [2-5]. Inflammation is a common component of many of these

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conditions, with the cellular mediators of inflammation also being involved in the pathophysiology of obesity, diabetes and metabolic syndrome. This provides an explanation for the wide ranging medicinal activities of *S. frutescens*. Macrophages are of particular importance to inflammation and immune responses. Two major macrophage sub-populations involved in the regulation of inflammation and immune responses have been defined: classically activated M1 macrophages, which stimulate inflammation, and alternatively activated M2 macrophages, which have anti-inflammatory activity.

Based on information collected from electronic databases including Science Direct, PubMed, Web of Science, Google Scholar, Springer Link, Wiley Online Library from 1965 to 2019 and published sources including short communications, reports, conference proceedings and theses, we review the influence of *S. frutescens* on the macrophage polarization states which play critical roles in insulin resistance (IR), obesity, and type 2 diabetes (T2D). We discuss the mechanisms by which the plant's phytocompounds may regulate macrophage function which, in turn, influences wider metabolic immunomodulatory processes, thus providing a rationale for its traditional use and the potential for future therapeutic use as an immune regulator.

We first describe the traditional preparation of extracts from *S. frutescens*, their use in indigenous medicine and the known phytocompounds found in aqueous and organic extracts. Next, we evaluate the observed roles of *S. frutescens* in reversing IR associated with T2D and its anti-inflammatory and immunomodulatory action, followed by the interaction between inflammation and metabolic disorders, the role of macrophages in these conditions and the potential mechanisms of action of *S. frutescens* extracts in the regulation of macrophage function.

2. The South African medicinal plant *Sutherlandia frutescens*

2.1. Taxonomy & botanical Information

Sutherlandia frutescens (L.) R.Br is also known as *Lessertia frutescens* (L.). The genus *Sutherlandia* belongs to the family Galegeae and is closely related to the genera Astragalus L and Lessertia DC. The plant is a prostrate to erect, perennial shrub, varying in height from 0.2-2.5m, with ovate-oblong leaves and red flowers, as shown in Fig. 1. The fruits are large, bladdery, papery pods with numerous seeds. Local names include cancer bush, sutherlandia, balloon pea and turkey flower in English and kankerbos, gansies, wildekeur and belbos in Afrikaans [5].

2.2. Traditional uses

Extracts of the plant *S. frutescens* are used in southern Africa to treat many conditions. Traditionally "two or three leafy twigs" are infused in boiling water to produce a tea given as a daily dosage. From the time of the San and Nama people in Southern Africa, *S. frutescens* has been used to treat wounds and fevers and, more recently, its use in the treatment of a number of conditions, including cancer,

Fig. 1. *Sutherlandia frutescens*, a traditional medicinal plant. Indigenous to South Africa, Lesotho, southern Namibia and south- eastern Botswana. Photographed by author in 2014.

diabetes, inflammation, back pain, stomach pain, eye diseases, skin diseases, influenza and HIV/AIDS has been reported [1-5].

2.3. Active compounds and pharmacological properties

Various bioactive compounds have been identified in *S. frutescens*, but the mechanism of action of these compounds is not well defined. Studies regarding the effects of *S. frutescens* on diabetes, cancer, HIV/AIDS and inflammation include investigation of known potentially active compounds [1, 6]. The phytocompounds derived from leaves of *S. frutescens* have been reviewed by van Wyk and Albrecht [5]. The compounds cyclitol and pinitol were first identified by Snyders [7], Viljoen [8] and Brümmerhoff [9]. Pinitol has been shown to act as an anti-diabetic agent, lowering blood glucose and increasing glucose uptake to support cell metabolism [10]. L-canavanine, a non-protein amino acid with anticancer and antiviral activity, is found in *S. frutescens* seeds [11]. Triterpenoid glycosides identified in the leaves of *S. frutescens* function as agonists of peroxisomal proliferator-activated receptors (PPAR) -α and -γ, enhancing lipid metabolism and reducing inflammation [12]. *S. frutescens* also contains γ-aminobutyric acid (GABA), an inhibitor of neurotransmission that reduces the pathophysiological effects of anxiety and stress [13]. GABA also inhibits tumour cell migration [14]. The anti-cancer action of *S. frutescens* is further indicated by the induction of apoptosis in cancer cells, including cervical carcinoma and Chinese Hamster Ovary cell lines following treatment with aqueous extracts of the plant [15].

Using an untargeted Triple TOF LC-MS/MS separation technique, Adefuye [16] analysed crude aqueous and organic *S. frutescens* leaf extracts and solid phase extraction fractions derived from these preparations. Multiple compounds were found in individual extracts, including 10 compounds with potential anti-diabetic activity, namely α-Pinene, Limonene, Sabinene, Carvone, Myricetin, Rutin, Stigmasterol, Emodin, Sarpagine and Hypoglycin B. Fu and colleagues [17] characterised two groups of compounds unique to *S. frutescens*, namely the cycloartane glycosides sutherlandiosides A to D; and the sutherlandins A to D, flavonol glycoside derivates of 3-hydroxy-3-methylglutarate. Gonyela and colleagues [18] also identified the cycloartane derivatives sutherlandioside A and sutherlandioside B, along with other unknown compounds in ethanolic extracts of *S. frutescens* leaves, using thin-layer chromatography. *S. frutescens* contains many other compounds including plant steroids, flavonoids, triterpenes, saponins and amino acids, none of which have been clearly identified as bioactive components [5, 19]. However, the full phytocompound composition has not been characterised. Both known and unknown components may have bioactive functions and potentially synergistic effects which could contribute to the different activities found in *S. frutescens* extracts produced from different plant parts and by differing extraction techniques.

S. frutescens has been shown to have no toxic effects in Vervet monkeys given a dose up to 80 mg kg^{-1} of dried *S. frutescens* powder [20], or in healthy volunteers given 400 mg of dried *S. frutescens* powder in capsule form twice daily for 24 weeks [21].

2.4. The role of *Sutherlandia frutescens* in the reversal of insulin resistance associated with type 2 diabetes

The anti-diabetic action of *S. frutescens* was indicated by Chadwick *et al*. [22], who treated Wistar rats fed a diabetogenic diet with an aqueous extract of *S. frutescens* leaves. They demonstrated that cellular glucose uptake was increased, indicating restoration of insulin sensitivity in the rats. MacKenzie *et al*. [23] further investigated the capacity of the aqueous extract of *S. frutescens* to prevent IR and T2D in male Wistar rats fed a diabetogenic high fat diet (HFD) for 16 weeks post weaning. After 12 weeks, the rats developed IR, confirmed by elevated plasma insulin and glucose, when compared to normal control rats fed a low fat diet (LFD). HFD rats gavaged with a human equivalent dose of the extract (0.05g kg^{-1} body weight per day) from one to 12 weeks, had a significantly lower mean body weight at 12 weeks than the HFD fed rats, similar to those of the LFD controls. Fasting plasma insulin and glucose levels were significantly lower in the *S. frutescens*-treated HFD group compared to untreated HFD-fed rats from week two to week

12, with the *S. frutescens*-treated group showing lowered HOMA-IR and QUICKI index, similar in value to the LFD control group [23]. However, there was no change in plasma triglyceride levels between the untreated and *S. frutescens*-treated HFD groups [23]. The same aqueous extract of *S. frutescens* used as a treatment for IR in 12-week old HFD rats gavaged for 4 weeks with a similar human equivalent dose of *S. frutescens* aqueous extract produced a normalization of total plasma cholesterol and lowered total lipid content in the liver. Rats treated with *S. frutescens* became more insulin sensitive, with increased glucose uptake, compared to the untreated HFD-fed rats. These findings correlate with those of Chadwick indicating that *S. frutescens* restores insulin sensitivity and reduces plasma free fatty acids in rats fed diabetogenic diet [22-25].

Williams [26] used an *in vitro* model of Insulin/ Fructose-induced IR in human Chang liver cells, in which they demonstrated that culture with high insulin and fructose concentrations resulted in reduced cellular glucose uptake and a consequent significant increase in glucose within the culture medium. Simultaneous treatment with 12.5 µg ml^{-1} *S. frutescens* prevented development of this IR, stimulated glucose uptake and reversed associated changes in lipid accumulation [27]. These *in vitro* findings correlated with the *in vivo* observations of IR and its prevention or reversal by *S. frutescens* in HFD rats [23, 24].

Williams [26] also analysed changes in expression of diabetes-related genes in IR and *S. frutescens*-treated Chang cells using the RT^2profiler™PCR Diabetes Array [27]. Many diabetes-related genes were down regulated in the IR culture, but up-regulated by treatment with *S. frutescens*, including the transcription factors hepatic nuclear factor 4-α (HNF4α), nuclear respiratory factor (NRF1) and CCAAT/enhancer binding protein α (C/EBPα); and the signal transduction factor dual specificity phosphatase 4 (DUSP4) [26]. *S. frutescens* also upregulated the glycolysis pathway and the pentose phosphate pathway by increasing expression of the sterol regulatory element-binding protein-1 (SREBP-1) and glucose-6-phosphate dehydrogenase (G6PDH) genes. Genes involved in glycogen degradation, and inhibition of glycogen synthase kinase 3 beta (GSK3β) were also upregulated [26], along with the insulin signalling pathway components encoded by the insulin receptor substrate 1 (IRS1), IRS2 and phosphoinositide 3-kinases (P13K) genes [27]. Hepatic cell cultures (HepG2 and Chang) similarly made insulin resistant by exposure to high concentrations of insulin and fructose and treated with aqueous, methanol or ethanol extracts of *S. frutescens* showed decreased gluconeogenesis, increased glucose uptake and decreased Tri-, Di- and Mono-acylglycerol accumulation, compared to IR controls not treated with *S. frutescens* [16]. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis indicated that the aqueous and organic *S. frutescens* extracts regulated expression of vesicle-associated membrane protein 3 (VAMP3), N-ethylmaleimide sensitive fusion (NSF) protein, mitogen-activated protein kinase 8 (MAPK8) and IRS1 at the mRNA level in the hepatic cells, all of which were down regulated in IR cultures [16]. VAMP3 and NSF are involved in vesicle-mediated transport of membrane impermeable molecules, between organelles and across the plasma membrane of cells [28], suggesting that *S. frutescens* has the potential to regulate glucose transporter (GLUT) proteins *via* the structural proteins NSF and VAMP3, thereby reversing IR [29]. Some confirmation of this was given by *in vivo* studies of *S. frutescens*-treated HFD-fed rats. *S. frutescens* reversed the down-regulation of NSF and VAMP3 mRNA and reverted the protein expression patterns of VAMP3, NSF and synaptosomal-associated protein (SNAP)-25 proteins in the liver of HFD rats towards the normal pattern, indicating changes in the transport of lipid-filled vesicles [29]. The influence of *S. frutescens* on vesicle transport has been shown in the c2c12 mouse muscle cell line, in which *S. frutescens* mimicked the effects of the insulin signalling pathway. This induced GLUT4 translocation and subsequent phosphorylation of the insulin receptor, which stimulated PI3K activity, leading to phosphorylation of Akt (protein kinase B) and activating the PI3K-Akt signal transduction pathway to promote cell survival and growth. Similarly, *S. frutescens* stimulated INS-1 rat pancreatic cell proliferation [30].

Thus *S. frutescens* has a demonstrated capacity to reverse IR and an anti-diabetic activity. The

pathophysiology of obesity, diabetes and the metabolic syndrome involves many cellular mediators of inflammation, in which macrophages play a key role; therefore the influence of *S. frutescens* extracts on macrophage activity in culture has been investigated.

2.5. The anti-Inflammatory effects of *Sutherlandia frutescens* in an *in vitro* mouse cell model

Lei *et al*. [31] used transcriptome analysis to investigate the influence of *S. frutescens* extracts on gene expression in the murine RAW 264.7 macrophage cell line. Time and concentration-dependent treatment of these macrophages with an ethanolic *S. frutescens* extract altered the expression of 547 genes. A low dose of 8 µg mL^{-1} ethanolic *S. frutescens* only down-regulated 2 genes and up-regulated 2 other genes after 24 hours treatment. However, cells exposed to treatment with a ten times higher dose of 80 µg mL^{-1} extract for 8 hours showed differential expression of 79 genes and, after 24 hours treatment, expression of 226 genes was modulated. Many of these differentially expressed genes were shown to participate in inflammatory signaling pathways, such as those involving nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) and MAPK [31]. RNA sequencing analysis in RAW 264.7 macrophages co-stimulated with and without bacterial lipopolysaccharide (LPS) and interferon gamma (IFNγ) indicated that the ethanolic *S. frutescens* extract altered the expression of 715 genes, with 117 being associated with immune function. In particular, the NFkB, MAPK and Janus kinase-signal transducer and activator of transcription proteins (JAK-STAT) signaling pathways were down regulated by treatment with the extract, both by an increase in expression of inhibitors, and by decreasing activators of the NFkB and MAPK signaling pathways. This regulation of pro-inflammatory signaling pathways led to reduction in the expression of the pro-inflammatory mediators, tumor necrosis factor (TNF) and interleukin 6 (IL6). However, expression of the cytokines IL1α and IL1β was increased [31]. These two cytokines have pleiotropic effects, influencing acute and chronic inflammation and autoimmune diseases [32]. A separate study of RAW 264.7 cells stimulated by LPS and treated with an ethanolic *S. frutescens* extract at concentrations between 50 and 200 µg mL^{-1} decreased the production of two key inflammatory mediators, reactive oxygen species (ROS) and nitric oxide (NO), in a dose-dependent manner [33]. A hot aqueous *S. frutescens* extract at a concentration of 200 µg mL^{-1} also reduced TNF-α and IL-6 levels induced by LPS, although not significantly [33]. These anti-inflammatory effects were further associated with diminished activation of the extracellular signal-regulated kinase (ERK), STAT1α and NFκB-dependent signaling pathways [34], thus indicating the potential for *S. frutescens* to influence the signalling pathways stimulating inflammatory responses in macrophages.

Both hot aqueous and ethanolic extracts of *S. frutescens* have been shown to significantly inhibit M1 pro-inflammatory macrophage functions and alter the M1 Cluster of Differentiation factor (CD) 86 marker expression pattern to the M2 (anti-inflammatory) CD206 pattern in RAW 264.7 macrophages, stimulated with LPS [33]. M1-type pro-inflammatory cytokines normally up-regulated by LPS activation were down-regulated, whilst the M2 cytokine profile remained unchanged. This action of *S. frutescens* was mediated through suppression of both the NFκB and MAPK pathways [35].

Thus, in this mouse cell culture model *S. frutescens* regulates, at the transcriptional and translational levels, expression of gene products involved in inflammatory responses and influences inflammatory signaling pathways, including those involving NFkB and MAPK.

2.6. Immunomodulatory effects *of Sutherlandia frutescens* in an human cell model

Findings from the murine cell culture models have been supported by analysis of human monocyte and macrophage cell models. Kisten [36] reported that an ethanolic *S. frutescens* extract at concentrations of 50, 500 and 5000 µg mL^{-1} regulated expression of the cytokines IL6, IL10 and IFNγ in human whole blood stimulated *in vitro* with LPS or phytohaemagglutinin. However, these effects were found to be blood donor related [36]. Ngcobo and colleagues [37], using ethanolic and aqueous extracts of *S. frutescens* to treat human peripheral blood mononuclear cells (PBMCs), showed that concentrations of ethanolic *S. frutescens*

extract over 500 µg mL^{-1} reduced the secretion of IL1α, IL1β and TNFα in PBMCs stimulated with either phytohaemagglutinin or camptothecin. However, ethanolic and aqueous *S. frutescens* extracts at a concentration of 2500 µg mL^{-1} can be cytotoxic to lymphocytes and therefore will affect their cytokine secretion [37]. Faleschini *et al*. [38] analysed the influence of hot aqueous and ethanolic extracts of *S. frutescens* and fractions of these extracts on the secretion of six cytokines, namely IL1β, IL6, IL10, IL12p70, IL8 and TNF, by HL60 neutrophil-like human leukemia cells co-stimulated with phorbol 12-myristate 13-acetate (PMA). No release of any of these six cytokines was detected when 25 µg mL^{-1} of the extracts or fractions were added to HL60 cultures without PMA co-stimulation. However, a fraction isolated from the ethanolic extract, together with PMA treatment, increased secretion of TNF and IL8. The hot aqueous extract and fractions, combined with PMA treatment, in contrast, decreased release of IL8, thus indicating differences in the action of the different phytocompounds present within these two extracts. PMA is recognised by pattern recognition receptors (PRR's) on the surface of the target cell that are linked to a response to bacterial LPS. PMA-binding stimulates expression of TNF and IL8, which attracts neutrophils, T cells and basophils to an inflammatory site. Thus, ethanolic extracts of *S. frutescens* possess immune boosting effects acting upon PMA-stimulated cells, whilst aqueous extracts have an anti-inflammatory action [38].

The immunomodulatory mechanism through which *S. frutescens* extracts resolve and regulate low-grade metabolic inflammation was further examined in the human THP-1 monocytic cell line, in which the monocytes were differentiated into pro-inflammatory macrophages using LPS [39]. Hot aqueous or ethanolic *S. frutescens* extracts at concentrations of 50, 100 and 200 µg mL^{-1} reduced expression of the M1 macrophage marker CD86. Following M2 induction with IL4/IL13, the M1 and M2 cell surface markers were reverted to baseline M0 unstimulated macrophage expression by both extracts [39]. These changes may be linked to decreased phosphorylation of members of the pro-inflammatory MAPK, p38 and NFκB signalling pathways and subsequent reduction in nuclear translocation of phosphorylated NFκB subunits, p65 and p50 (Fig. 2). An *S. frutescens*-mediated decrease in this nuclear transcription factor was shown to be associated with a down-stream decrease in expression of the pro-inflammatory gene products cyclooxygenase 2 (COX2), TNFα, IL-6 and macrophage inflammatory protein-1 β (MIP-1β) [39] (Fig. 2). The MAPK pathway that signals cell proliferation, differentiation and cell survival was regulated by both *S. frutescens* extracts. The observed initial reduction in phosphorylation of ERK1/2 following treatment with *S. frutescens* may result in apoptosis of early responding pro-inflammatory monocyte/macrophage cells. A later increase in phosphorylation of ERK1/2 could, *in vivo*, stimulate proliferation of phagocytic macrophages, which are recruited to inflamed areas to resolve inflammation [39] (Fig. 2).

Fortuin-Seedat [39] observed Akt-independent regulation of MAPKs, ERK1/2 and p38 by ethanolic and hot aqueous *S. frutescens* extracts. In addition, Akt-dependent (and/or independent) regulation of the NFkB pathway was shown, which could indicate regulation of the GSK3β signalling pathway [39] (Fig. 3). This led to the proposal that *S. frutescens* extracts regulate the down regulation of nuclear factor kappa B kinase (IKK) pathway and/or the GSK3β signalling pathway, *via* inhibition of the Akt-independent IKK pathway and activation of the Akt-dependent GSK3β signalling pathway to exert anti-inflammatory effects [39] (Fig. 3). The signalling may involve negative feedback action by GSK3β, which plays a central role in regulating inflammation that is associated with pathophysiological conditions such as IR, T2D and obesity [41].

2.7. Immunomodulatory effects of phytochemicals isolated from *Sutherlandia frutescens*

Differing and sometimes contradictory actions of *S. frutescens* extracts have been recorded, which are likely to reflect the phytochemical composition of extracts prepared from plant materials derived from *S. frutescens* plants grown in several different geographic locations in southern Africa. This variability in growth location will generate chemical heterogeneity between plant populations, reflecting local sub-populations of plants and differences in the growth conditions. In addition, extracts may be produced from plants at differing times of the year

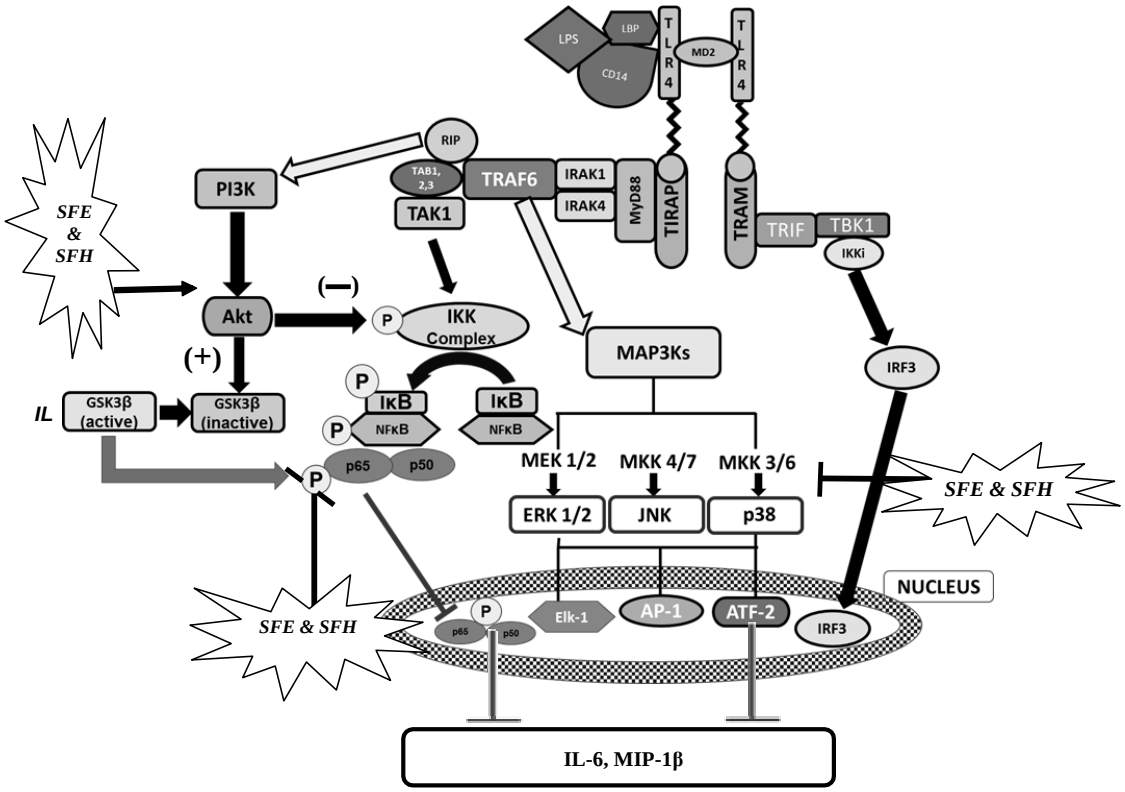

Fig. 2. The immunomodulatory effects of ethanolic and hot *S. frutescens* extracts on LPS-induced THP-1 macrophage cell line (modified from [40]). LPS activates three distinct pathways during an inflammatory response. These pathways include the MAPK pathway, the NFκB pathway and the P13K/Akt signal transduction pathway. Novel immunoregulatory mechanisms through which *S. frutescens* may resolve and regulate inflammation are indicated by - (inhibitory) and + (stimulatory). (+) and (-) symbols represent the possible Akt regulatory mechanism used by the hot aqueous *S. frutescens* extract (SFH) and the ethanolic extract (SFE).

and hence in different stages of their growth cycle. Finally extracts may be prepared from stems, leaf, flower, or the whole plant [5]. Furthermore, some researchers have used laboratory micropropagation of plants, which influences the phytochemical composition of the preparations [44]. Thus, variations in the reported action and phytochemical content of extracts may occur. Key differences in identified metabolites, such as flavonoids, triterpenes, saponins and steroids [5], will influence the medicinal bioactivity of *S. frutescens* extracts. Phytochemicals unique to *S. frutescens* have been isolated to assist in standardization and to assess the quality of the plant extract. These are quercetin, terpenoids/ cycloartenol glycosides (sutherlandiosides A to D) [17, 45] and kaempferol-derived flavonoids (sutherlandins A to D) [17, 19].

Immune activation by *S. frutescens* has been demonstrated by several groups. Pectin-type polysaccharides isolated from *S. frutescens* were reported by Zhang and co-workers [46] to stimulate innate immune cells *via* complement fixation, in an activation cascade that will enable removal of microorganisms and damaged cells. Lei *et al*. [47] observed an increase in the production of NO, pro-inflammatory cytokines and ROS following treatment of leukocytes with a polysaccharide-enriched fraction from *S. frutescens*, [47]. They postulated that the plant polysaccharides stimulated the cells by binding to surface receptors, such as the toll-like receptor-4 (TLR4), CD14, dectin-1 and mannose receptor, which are key to innate immune signaling [47]. Other biological studies of the activity of *S. frutescens* extracts, fractions and compounds, including sutherlandioside A and sutherlandioside

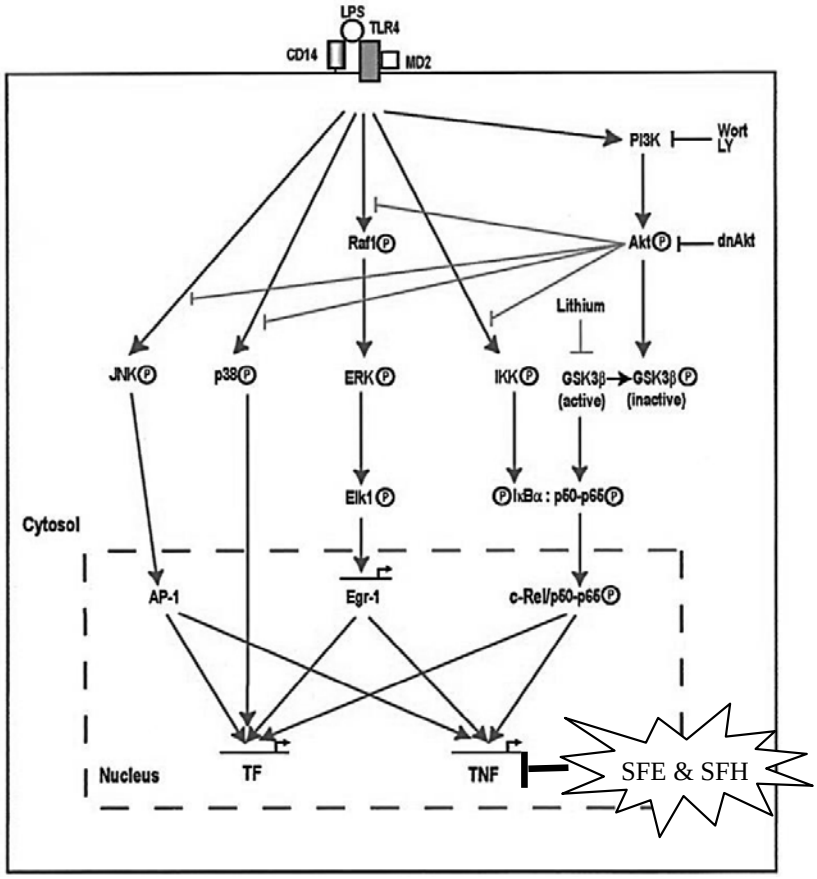

Fig. 3. Activation of the PI3K pathway in monocytic cells limits LPS-induced TNFα and TF gene expression. Binding of LPS to the CD14 and TLR4/MD2 complex activates the PI3K-Akt signalling pathway. Akt directly or indirectly inactivates the MAPK (ERK1/2), p38 and JNK and the NFkB pathways by regulating upstream kinases, including Raf-1, MEKK3, and IkB kinase. LPS activation of PI3K/Akt also inactivates GSK3β, which leads to reduction of the trans-activational activity of p65 NFkB. Akt-dependent inactivation of any of these pathways can, in turn, reduce the activation of the transcription factors NFkB, AP-1 and Egr-1. NFkB, AP-1 and Egr-1 cooperatively regulate TNFα and TF gene expression. In animal models, TNF and tissue factor (TF) are expressed by adipose tissue and upregulated in obesity, with IR as a modulator (modified from [42, 43]).

B, on cytokine expression, indicated inhibition of the expression of IL-10 and TNFα [18]. Thus a number of phytocompounds within extracts of *S. frutescens* have the capacity to influence immune responses, inflammation and related metabolic dysregulation, both positively and negatively [44].

3. Inflammation and metabolic syndrome

In this section we discuss how *S. frutescens* may influence clinical aspects of the wider metabolic syndrome by acting upon underlying inflammatory events and macrophage activation. Firstly, we will describe metabolic syndrome and the involvement of inflammation in this group of disorders.

The World Health Organisation (WHO) defines obesity, IR, dyslipidaemia and hypertension as key components of the metabolic syndrome [48]. These conditions share common mediators, pathways and pathophysiological mechanisms [49]. The National Cholesterol Education Program (NCEP): Adult Treatment Panel III (ATP III) further states that metabolic syndrome is caused equally by glucose intolerance, hypertension, dyslipidaemia and obesity [50-52].

Obesity is increasing in prevalence globally, associated with a western lifestyle. Negative factors associated with such a lifestyle include prolonged psychological stress; intake of energy dense food, rich in fat, but poor in micronutrients; low levels of physical activity; and disrupted sleep patterns [53, 54]. Previously, adipose tissue was considered to provide mechanical protection, thermal insulation, and to serve as an excess energy storage site. Now it is considered to be a multi-depot organ that maintains metabolic homeostasis, as well as an active endocrine organ secreting adipokines, which regulate food intake, metabolism, insulin sensitivity and immune responses [55, 56]. The characteristics of adipose depots differ, depending on location and immune cell content. This, in turn, influences their role in metabolic disease, with visceral adipose tissue being particularly linked to increased susceptibility to metabolic syndrome [53]. Increasing the size and number of adipose depots alters the adipose microenvironment, enhancing recruitment of immune cells, such macrophages, into the tissue and increasing activation of both the resident and infiltrating cells. This, in turn, increases pro-inflammatory adipokine production and alters the interactions between recruited immune cells and local adipocytes. The resultant inflammatory response indirectly links inflammation to development of metabolic syndrome.

Inflammation is a physiological response to harmful physical, chemical and biological stimuli that involves coordinated interaction by multiple immune cells and their products to re-establish homeostasis. The identity and effects of these products depend on the initial stimulus [54]. Many mediators of inflammation are also involved in obesity and diabetes, although in these conditions, they are not triggered by biological infection or injury. Few, if any, of the classical features of inflammation, such as swelling, pain and fever have been observed in obese or diabetic individuals. In these disease states an initial inflammatory response results in secretion of excessive levels of cytokines and acute-phase reactants, which trigger the activation of further inflammatory signalling pathways [57].

Thus, the accumulation of adipose tissue caused by over-nutrition in obesity, leads to locally altered metabolism, decreased blood flow through the tissue, increased secretion of pro-inflammatory cytokines by hypertrophic adipocytes and migration of cells of the adaptive and innate immune system into the tissue [58, 59]. This local inflammatory response and the resultant systemic inflammatory response impair pancreatic beta (β)-cell functions, reduce insulin signalling and may contribute to later development of T2D. Obesity is also associated with oxidative stress and Toll-like receptor activation in adipocytes [59], probably as a consequence of fatty acid accumulation [59-63]. Oxidative stress, endoplasmic reticulum stress [64] and amyloid deposition in the pancreas [65] are all linked with IR and resultant β-cell dysfunction. Associated with this is lipid deposition in the muscle, liver and pancreas, leading to lipotoxicity and glucotoxicity [66], all of which result from over-nutrition [67, 68]. Each of these cellular stresses induces, or exacerbates, inflammation and impact on the body's response to inflammation [69, 70].

The pathological inflammatory state found in metabolic disease has the physiological counterpart of low-grade and chronic inflammation. In this state, the same inflammatory mediators are present in the absence of infection or injury, and are associated with tissue dysfunction, causing an imbalance in physiological systems not directly related to host defence or wound-healing [71]. The causes of inflammation and the extent to which it contributes to an increased risk of chronic metabolic disorders are still unknown [72].

Whilst inflammation is a protective mechanism central to immune function, chronic and persistent low-grade inflammation contribute to the pathology of T2D, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome and cardiovascular disease [73, 74]. This low-grade, metabolically activated inflammation forms a characteristic intermediate between a non-inflammatory state and the inflamed state [71]. It is triggered both acutely and chronically by excessive nutrient intake [75], and non-nutritive food components which modulate low-grade inflammation [72, 76]. Classical inflammation, in contrast, is triggered by injury and is characterised by local swelling, redness, heat and pain that is usually of short duration and quickly resolved, stimulating wound healing at the injured site. However, the consequences of prolonged classical inflammation are not beneficial [75].

Therefore, it is important to distinguish classical inflammation, associated with response to injury, from low-grade chronic inflammation, and from metabolically triggered 'meta-inflammation' [71, 75].

New therapies are needed to treat obesity and T2D, including treatment of the chronic inflammation associated with these diseases. Investigation of medicinal plants as alternative therapies to pharmacological drugs continues to flourish, due to decreases in efficacy and availability of pharmacological drugs, increases in cost, side-effects and contraindications which make the use of natural medicinal plants a treatment of choice for many people [77].

4. The monocyte/macrophage lineage in inflammation

Cells of the monocyte/macrophage lineage form an essential component of the innate immune system due to their phagocytic action and are also antigen-presenting cells which act as mediators of the adaptive immune response [78]. These cells are a heterogeneous family involved in the recognition and clearance of pathogens and damaged or dead body cells. The tissue microenvironment, such as the small intestine, adipose tissue, or alveolar space, may regulate the functional properties of macrophages [79]. As a result of their multiple actions, the monocyte/macrophage lineage plays a critical role in the initiation and resolution of inflammation, directly through phagocytosis and activation of ROS, and more indirectly by the secretion of inflammatory cytokines and the activation of lymphocytes in the adaptive immune response [80, 81].

Circulating monocytes in the blood are recruited to sites of tissue injury and inflammation. There they contain and remove infective organisms and stimulate repair of damaged tissue [82]. Within the body tissues monocytes differentiate into either dendritic cells or macrophages [83]. This migration and differentiation is stimulated, and the exact differentiation pattern determined, by components of the inflammatory environment that interact with pathogen-associated molecular pattern (PAMP) receptors (PRRs) on the cell surface of monocytes. However, local accumulation of cells of the monocyte/macrophage lineage can be harmful and aggravate varied diseases such as arthritis, multiple sclerosis and atherosclerosis [83-85].

Macrophages are resident in almost every organ. Their differentiation patterns reflect exposure to factors such as colony stimulating factor-1 (CSF-1), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF) and other cytokines, which enable them to adapt to environmental stimuli [86]. The two main activation states are the classically activated type 1 macrophage (M1) and the alternatively activated type 2 macrophage (M2) (Fig. 4) [87, 88].

4.1. Classic and alternative macrophage activation and polarization

Classically activated M1 macrophages are effector macrophages in cell-mediated immune responses. These cells are also activated by innate stimuli that form part of stress responses [90]. Macrophages are classically activated by exposure to two cytokine signals. The first signal involves IFN-γ, produced by cells in the innate immune response (Fig. 4), which stimulates responsive macrophages to produce superoxide anions and oxygen or nitrogen radicals to enhance pathogen killing and to release pro-inflammatory cytokines to amplify the response [91]. This macrophage activation is short-lived; therefore further stimulation by IFN-γ produced by antigen-specific helper T cells (Th1) of the adaptive immune response is needed to maintain the activation state and provide a stable immunological defence [92] (Fig. 5a).

TNF is the next signal from Th1 cells. It induces increased microbicidal and tumoricidal activity in the M1 macrophage population, with high secretion of pro-inflammatory cytokines, including IL-1, IL-6, IL-12 and IL-23 [92, 93], (Fig. 4 and Fig. 5a). These cytokines activate Th17 cells, a second helper T cell sub-population required for an effective immune response. However, activation of pro-inflammatory cytokines must be controlled since excessive immune activation can cause extensive tissue damage [92].

Alternatively-activated, or wound healing, M2 macrophages develop from an activation pattern distinct from the classically activated macrophages. M2 macrophages are divided into an M2a sub-group, activated by cytokines IL-4 or IL-13; an M2b sub-group activated by binding of ligand to their Fc-γ receptors in the presence of TLR stimulus; and the M2c sub-group which have deactivation

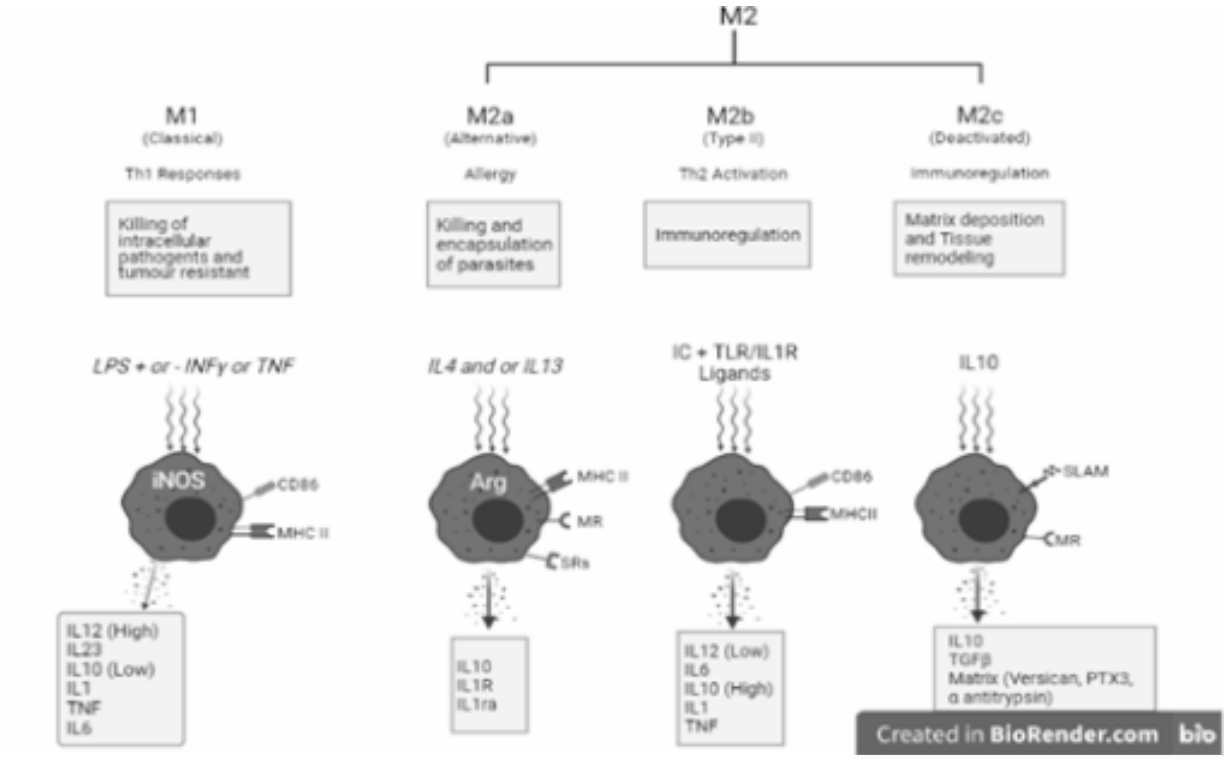

Fig. 4. M1-M2 macrophage model, derived from [89], indicating receptors and key signalling mediators in signalling pathways and immune responses associated with macrophage activation.

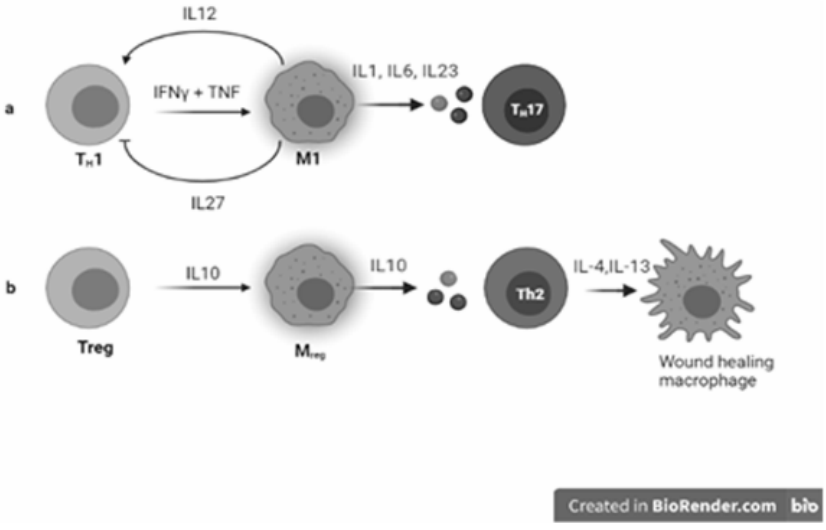

Fig. 5. (a) Interaction between macrophages and T helper cells. Interferon-gamma (INF-γ) produced by T helper 1 (Th1) cells in combination with tumour-necrosis factor (TNF) from antigen-presenting macrophages (APCs) give rise to M1 Macrophages. **(b)** Production of interleukin (IL)-10 from regulatory T cells (Treg) stimulates regulatory macrophages, which are APCs, to activate Th2 cells. Production of IL-4 and IL-13 by Th2 cells promotes the development of M2 macrophages (wound-healing) [92].

programs elicited by IL-10 or Transforming Growth Factor (TGF)-β [92, 94] (Fig. 4). We propose that *S. frutescens* influences macrophage activation patterns to stimulate resolution of inflammation and wound healing. We also suggest that different extracts of *S. frutescens* and their purified sub-fractions that contain differing combinations of phytochemicals will provide differential regulation of the M1 and M2 phenotypes.

4.1.1. Pro-inflammatory M1 macrophage stimulation *via* bacterial endotoxin activation

In this section we describe the characteristics and stimulatory mechanisms of M1 macrophages. Bacterial LPS, an activator of M1 macrophages, induces production of pro-inflammatory cytokines, including TNF-α, IL-1, IL-5, IL-8, IL-12 and IL-23, and pro-inflammatory mediators, including platelet-activating factor, prostaglandins, NO and reactive oxygen intermediates (ROI) [90, 95]. These inflammatory agents prevent the growth and spread of infective pathogens, but their dysregulated, excessive production can result in tissue damage and systemic complications, including microcirculatory dysfunction and septic shock [95]. LPS activates or deactivates macrophages *via* the LPS receptor complex, which is made up of three proteins: the 55kDa glycoprotein CD14, TLR4 and the myeloid differentiation protein-2 (MD-2).

In circulation, LPS in a bacterial cell wall binds to serum LPS-binding protein (LBP). This links the bacterium to monocyte/macrophage and myeloid cells *via* the cell membrane protein CD14, which then aids in the transfer of LPS to the TLR4/MD-2 receptor complex, modulating recognition of LPS by the cell [95]. TLR4 signalling is divided into either a MyD88-dependent pathway, or a MyD88-independent pathway involving the Toll/ IL-1R domain-containing adaptor-inducing IFN-β (TRIF)-dependent pathway. The MyD88-dependent pathway appears to induce pro-inflammatory cytokine expression, whilst the MyD88-independent (TRIF) pathway stimulates expression of Type 1 interferons and interferon-inducible genes [96].

Macrophage stimulation by the MyD88-dependent pathway (Fig. 6) activates IL-1 receptor-associated kinase 4 (IRAK4), which, along with IRAK1, activates the adaptor protein, TNF receptor-associated factor 6 (TRAF6). TRAF6 then activates transforming growth factor-β-activated kinase 1 (TAK1), which in turn activates the Inhibitor of kappa (κ) light chain gene enhancer in B cells (IκB) kinase (IKK) and MAPK pathways to regulate gene expression [96].

Regulation of gene expression occurs *via* phosphorylation and degradation of IκB kinase by a complex of IKKα, IKKβ and IKKγ enabling the translocation of NFκB into the nucleus, where it controls the expression of pro-inflammatory cytokine genes. Pro-inflammatory cytokine gene expression is also induced following activation of the MAPK pathway, with induction of c-Jun Component Activator Protein-1 (AP-1). In addition, IκB zeta (ζ) and the transcription factor interferon regulatory factor 5 (IRF5) are rapidly induced (Fig. 6) [96].

The MyD88-independent TRIF pathway (Fig. 7) is important to activate transcription factor IRF3 and is involved in later activation of NFκB and MAPK, *via* interaction between the C-terminal region of TRIF and the serine/threonine kinase receptor-interacting protein (RIP) 1 [96].

In addition, TRIF (Fig. 7) also interacts with TRAF3, which associates with TRAF family member-associated NFκB activator (TANK), TANK binding kinase 1 (TBK1) and IKKi to dimerize IRF3 and enable its translocation into the nucleus, where both NFκB and IRF3 induce transcription Type 1 interferons (IFN-α and IFN-β) and other target genes (Fig. 7) [96].

4.1.2. Anti-inflammatory M2 macrophage activation and polarization

We now discuss the anti-inflammatory M2 macrophage phenotype. Polarization of M1 to M2 macrophages is a dynamic process, with the phenotype being reversible under different physiological and pathological conditions [97]. The M1 macrophage phenotype is driven by IFNs and TLR signalling, which activate the IRF/ STAT1 signalling pathway. However, the IRF/ STAT pathway is also activated by STAT6 signalling, involving IL-4 and IL-13, which directs macrophage activity towards the M2 phenotype. LPS activation of NFκB and IRF family members by TLR4 and other TLR receptors can stimulate macrophage activation into either the M1 or the M2 state. A shift in the normal balance of M1 to M2 polarization

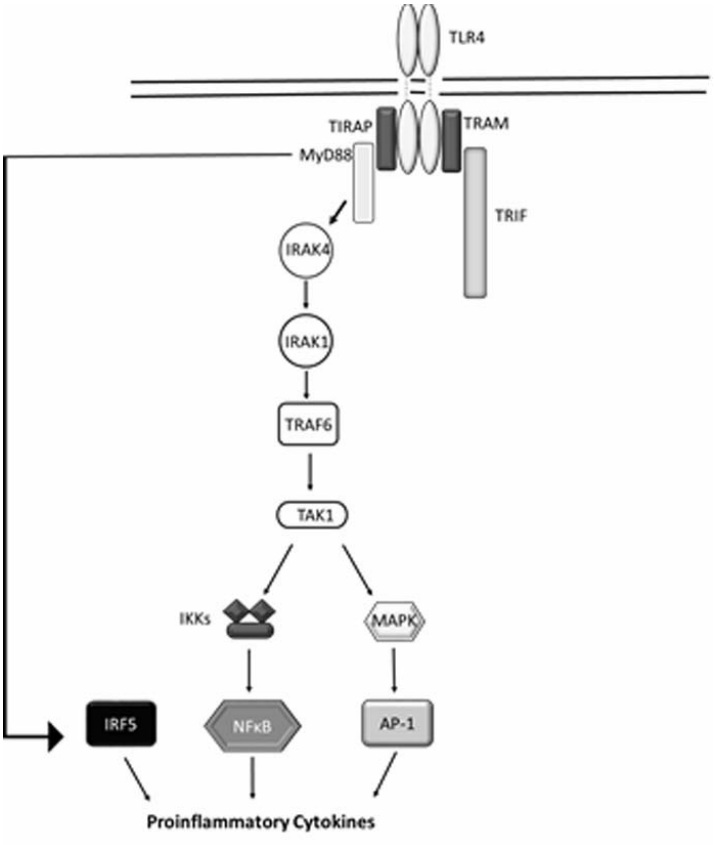

Fig. 6. The MyD88-dependent pathway (derived from [96]).

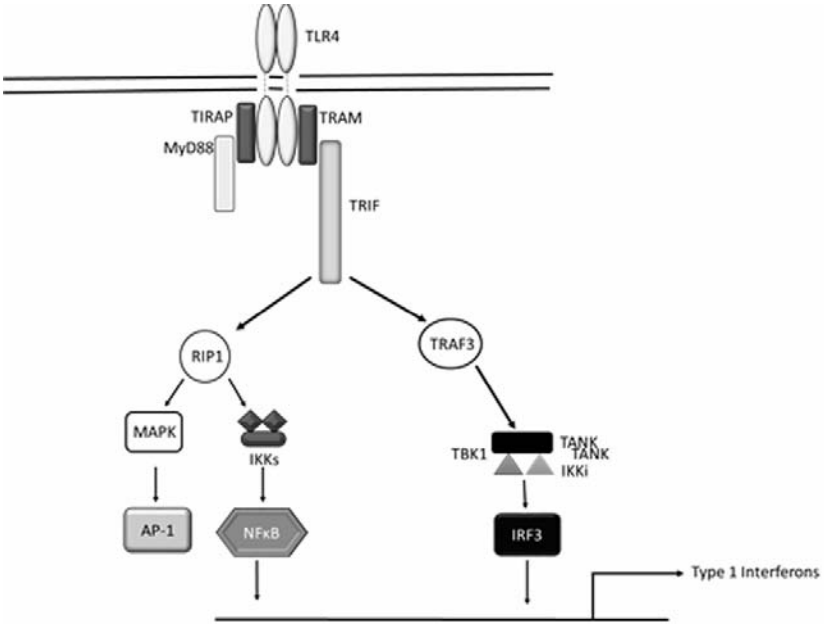

Fig. 7. The MyD88-independent (TRIF) pathway. TRIF signals induction of Type 1 interferon expression by recruiting TRAF3 and RIP1 [96].

characterises various diseases, in which the classically activated, pro-inflammatory M1 macrophages initiate and sustain chronic inflammation that M2 macrophages are unable to resolve [98]. Fortuin-Seedat [39] has shown that *S. frutescens* leaf extracts can influence macrophage polarisation *in vitro*. Understanding of the action of *S. frutescens* extracts upon the regulatory mechanisms involved in the phenotypic switch between M1 and M2 macrophage function will assist in developing new therapeutic interventions to re-establish homeostatic regulatory function between these polarized states.

4.1.2.1. M1 to M2 macrophage switch *via* the IRF/STAT signalling pathway

The IRF/STAT signalling pathway is central to controlling M1 to M2 macrophage polarization. It has been proposed that switching from a MyD88-dependent pathway to a MyD88-independent TRIF/TLR4 pathway could shift the phenotype of macrophages from pro-inflammatory action to anti-inflammatory action (Fig. 8) [90].

Polarization of macrophages to a M2 phenotype is mainly driven by the cytokines IL-4, IL-13 and IL-10 [99]. The inducible suppressor of cytokine signalling (SOCS) protein regulates this polarisation *via* STAT signalling to limit inflammatory responses; however cytokine signalling mechanisms can both induce and inhibit activity of SOCS, depending on the cytokine involved [100]. Thus, activation of STAT6 by IL-4 or IL-13 binding to the IL-4 receptor α (IL-4Rα) (Fig. 8) polarizes macrophages to a M2 phenotype and IL-10 binding to IL-10R activates STAT3 to stimulate development of a M2c state (Fig. 8) [90, 101, 102].

IL-4 and IL-13 primarily serve as anti-inflammatory molecules with a range of activities that regulate inflammatory responses [103]. Stimulation of IL-4 and IL-13 secretion by T cells involves different mechanisms [104], but these cytokines share biological functions, with both reducing production of IL-1 and TNFα to limit inflammation, and upregulating several monocyte/ macrophage markers, including IL-1Rα, mannose receptor (CD206), Dectin-1, YM1 and Fizz-1 (found in inflammatory zone 1 or RETNLA) [105, 106].

IL-4 signalling requires IL-4 to bind to the IL-4α receptor, forming a complex which binds to a secondary receptor, either IL-2Rγc (a type 1 IL-4 receptor) or IL-13Rα1 (a type 2 IL-4 receptor) (Fig. 9a and b). Formation of the complete receptor complex induces a conformational change that activates intracellular signalling molecules, with differing STAT involvement to induce expression of M2 genes [101, 107].

IL-13 bound to IL-13Rα1 recruits IL-4Rα into a functional receptor complex also favouring M2 macrophage polarization (Fig. 9a). Jak kinases, associated with IL-2γc (Jak3), IL-4Rα (Jak1), or IL-13Rα1 (Tyk2, Jak2) (Fig 9a and b), auto- and cross-phosphorylate each other, causing their activation [107]. Signalling from the fully assembled IL-13 receptor complex activates STAT6, STAT3 and STAT1 transcription factors, *via* the type 2 IL-4Rα receptor, whilst IL-4 either similarly activates STAT6, STAT3 and STAT1, or activates only STAT3 and STAT6 *via* the type 1 complex. Once activated, the STAT molecules homodimerize and translocate to the nucleus, where they induce STAT responsive genes (Fig. 9a and b) [107].

4.2. Inflammatory mediators influencing macrophage activation and polarization

Inflammation involves a series of dynamic responses in which macrophages act as control switches for the immune system, to balance pro- and anti-inflammatory responses, and to remove viruses, bacteria, damaged tissue, apoptotic cells and tumour cells [108]. Macrophages recruited from the circulation by inflamed cells or damaged tissue secrete signalling molecules and mediators, including vasoactive amines, bradykinin, eicosanoids, free radicals, cytokines and chemokines, which contribute to the inflammatory response [109].

The main pro-inflammatory cytokines are interferons, interleukins, CSF and TNFα and β, with IL-1 (α and β) and TNFα being particularly active in acute inflammation. Cytokines involved in chronic inflammation are divided into two groups. The first group mediates humoral responses, and includes IL-4, IL-5, IL-6, IL-7, and IL-13, while the second group mediates cellular responses, and includes IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, IFNs, TGFα and TNFα and β. Differentiation of T lymphocyte sub-sets and activation of macrophages themselves, cytotoxic T cells and NK cells to destroy

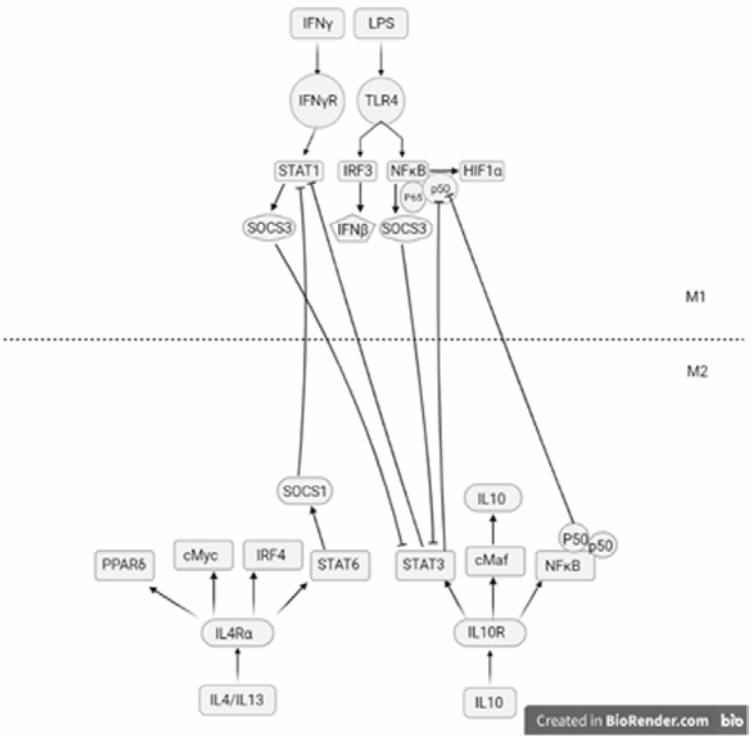

Fig. 8. Mechanisms involved in polarization of macrophages [90] Outline of the major regulatory pathways of macrophage M1 to M2 polarization.

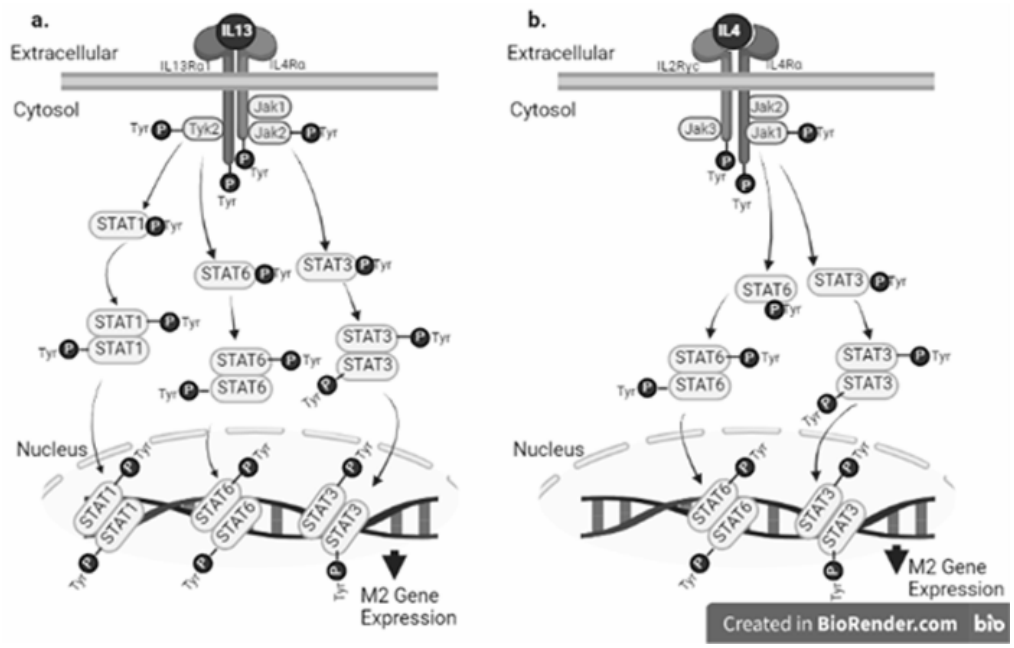

Fig. 9. The binding and activation of the common receptor IL-4 receptor α by both IL-13 (a) and IL-4 (b). Different Jak kinases are associated with these receptors and activated via tyrosine phosphorylation in response to IL-13 or IL-4 stimulation [103].

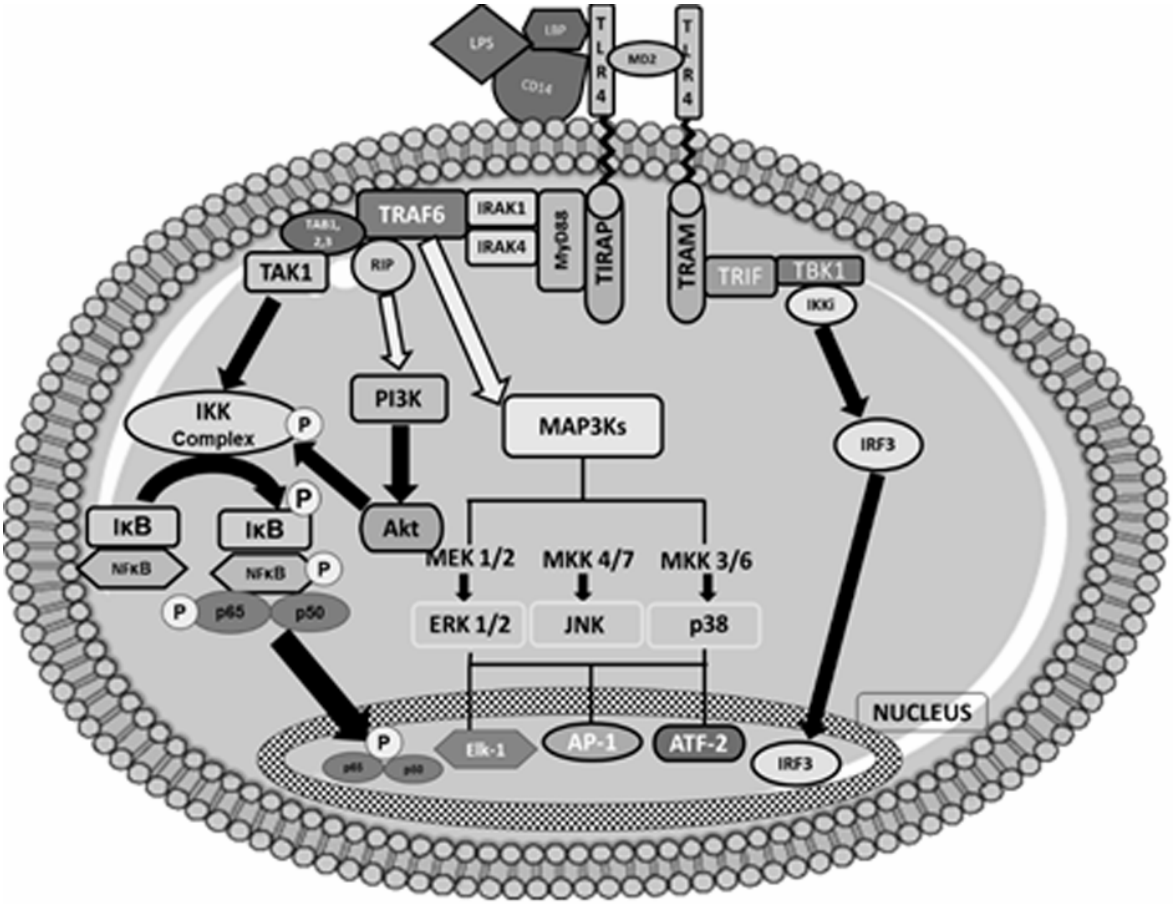

Fig. 10. MAP3Ks signalling pathway stimulated by bacterial endotoxin binding to TLRs [40].

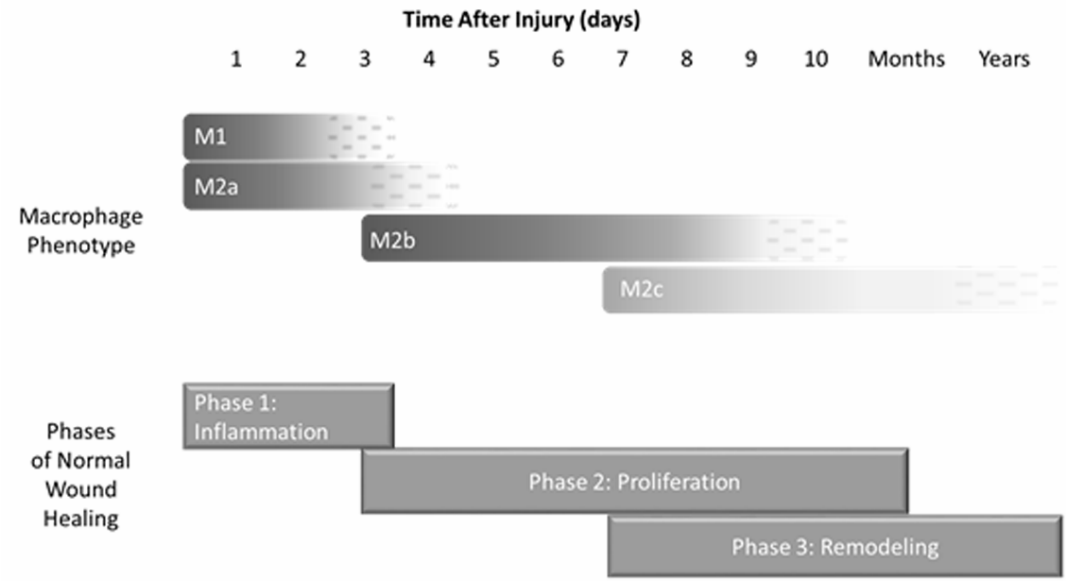

Fig. 11. Three phases of normal wound healing in the skin and muscle through a sequence of M1 to M2c macrophage activation, as proposed by Gensel and Zang [135].

intracellular pathogens are all regulated by these cytokines [110, 111].

Chemokines are a subclass of chemotactic cytokines that recruit cells of the innate and adaptive immune system to sites of infection or damage. Two important molecules involved in monocyte/macrophage attraction to inflammatory sites are macrophage inflammatory protein (MIP) -1 and dendritic cell-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing non-integrin (DC-SIGN).

MIP-1 is a chemokine induced by various pro-inflammatory stimulants that functions in both acute and chronic inflammatory responses at sites of infection or injury [112]. MIP-1 binds with high affinity to cell surface G-protein chemokine receptors to regulate the migration of monocytes and dendritic cells from the circulation, between the vascular endothelial cells, and into the inflamed tissue [112]. The resultant MIP-1/receptor complex triggers a cascade of intracellular changes involving PI3K pathway activation, activation of phospholipase C and Ca^{2+} influx, leading to chemotaxis, mediator release and phagocytosis [112, 113]. Other signal transduction pathways, such as the MAP kinase and the JAK/STAT signalling pathways, are also activated [112].

DC-SIGN is a cell surface receptor on dendritic cells, M2 macrophage subpopulations, and tissue resident macrophages in the placenta, lung alveoli and adipose tissues [114]. DC-SIGN mediates interaction between dendritic cells and naïve T lymphocytes *via* the intercellular adhesion molecule (ICAM)-3 [115] and dendritic cell trafficking *via* endothelial ICAM-2 [116]. DC-SIGN expression is increased when IL-4 stimulates the differentiation of monocytes into macrophages in the anti-inflammatory M2 macrophage activation pathway. Pro-inflammatory signals, in contrast, including IFN-γ reduce the inductive effect of IL-4 on DC-SIGN expression [117].

Thus *S. frutescens* may have immune modulating effects through the regulation of one or more of these upstream activators. The observed dual activity of *S. frutescens* through different extracts and preparations indicates its potential to interact with signalling molecules that are part of interacting intracellular signalling pathways. Lei and colleagues [47] propose that this regulatory activity may be the result of plant polysaccharides binding to one or more cell surface receptors involved in monocyte/macrophage differentiation and activation. Furthermore, *S. frutescens* regulates expression of genes encoding proteins involved in vesicle-mediated transport of membrane impermeable molecules, which could modulate the cell surface expression of important receptors and adhesion molecules [16, 27, 29, 30].

4.2.1. Intracellular signalling pathways

The key intracellular signalling pathways controlling macrophage activation are the MAPK, IKK, and PI3K pathways, with interactions involving Akt that regulate activation and nuclear translocation of transcription factors NFκB, ERK, JNK and p38. These pathways are stimulated by several agonists to modulate the activation pattern.

MAPKs are activated by TLR signalling *via* cytoplasmic Toll and IL1 receptor (TIR) domains, interacting with the TIR domain-containing adaptor protein (TIRAP), following agonist stimulation (Fig. 10). Ligand binding to TLR4 recruits IRAK1 to the TLR4 complex following interaction with MyD88 and IRAK-4 [118] (Fig. 10). IRAK-4 then forms a complex with IRAK-1, IRAK-2 and the E2 ubiquitin ligase TRAF6, activating both the NFκB and MAPK kinase (MAP3K) signalling pathways [119].

The NFκB signalling pathway is activated by MAP3K-TAK1 association with TAK1-binding protein 1 (TAB1) and the ubiquitin-binding proteins TAB2 and TAB3. This recruits TAK1 to the TRAF6 complex and stimulates TAK1 kinase activity [120], facilitated by the regulatory subunit (IKKγ) of the IKK complex and the catalytic subunits IKKα and IKKβ. TAK-1-mediated phosphorylation of IκB kinase (IκB) induces phosphorylation, cleavage and activation of NFκB, leading to nuclear translocation of the NFκB subunits, p65 and p50 [120, 121] (Fig. 6 & Fig. 10).

Activation of the MAP3K pathway *via* phosphorylation through TRAF6 activates other members of the MAPK family, including p38, the extracellular receptor activated kinases 1/2 (ERK 1/2), and c-jun-N-terminal kinase (JNK) [118]. LPS treatment has been shown to activate ERK in macrophages [122], but not in dendritic cells [123]. Both ERK and p38 are involved in

macrophage maturation [124], with ERK promoting maturation of macrophages, whilst p38 impairs dendritic cell development from human monocytes [125]. ERK enhances cell proliferation, whereas p38 and JNK are induced by cytokines and stress responses to mediate differentiation (associated with reduced proliferation) and cell death [126]. Activation of MAPKs therefore leads to changes in monocyte/macrophage cell proliferation, differentiation and regulation of the synthesis and release of pro-inflammatory cytokines, *via* production of transcription factors, including activating transcription factor-2 (ATF-2), ETS domain-containing protein-1 (Elk-1) and AP-1 (Fig. 10). Such activation of pro-inflammatory transcription factors further accelerates the process of inflammation [110] (Fig. 8).

Bacterial LPS activates macrophages *via* TLR binding and the PI3K pathway. The activated TLR binds to PI3K, which phosphorylates phosphatidylinositol, catalysing the formation of the second messenger phosphatidylinositol 3,4,5-triphosphate. This then activates downstream molecules, including Akt, which are important to cell survival, mitogenesis and cell migration [127, 128]. Activated Akt dissociates from the plasma membrane and stimulates IKK activity by phosphorylation of the IKKα subunit. The IKK complex then phosphorylates IκB kinase and the p65 subunit, enhancing activation of NFκB. Negative feedback by high amounts of non-degradable phosphorylated IκB kinase inhibits further production of NFκB [129] (Fig. 10).

The PI3K/Akt signalling pathway acts to negatively regulate acute inflammatory responses induced by LPS *in vitro* and *in vivo* [128]. Consequently, inhibition of this pathway enhances LPS-induced activation of the transcription factors NFκB, AP-1 and Egr-1, leading to increased TNFα expression [42], and activation of the PI3K/Akt pathway potentially suppresses LPS-induced pro-inflammatory responses [130].

The potential for *S. frutescens* to influence these signalling pathways has been demonstrated. Camille and Dealtry [33] found that *S. frutescens* mediated the inhibition of LPS-activation of RAW 264.7 murine macrophages to the M1 phenotype, and stimulated development of the M2 phenotype. Both Camille and Dealtry [33] and Lei *et al*. [34] showed that ethanol extracts of *S. frutescens* inhibited NFκB activation in this model system. These two groups also independently demonstrated that a hot aqueous extract [33] and a polysaccharide-enriched fraction of an aqueous extract [34] inhibited the inflammatory signalling of LPS to induce expression of responsive genes encoding ROS, NO, pro-inflammatory cytokines [33, 34] and COX2 [33]. Lei *et al*. [31] later identified over 100 genes regulated by inflammatory signaling pathways involving the NFkB and MAPK pathways that are differentially expressed following *S. frutescens* treatment [31]. This activity also extends to human cells. Secretion of certain pro-inflammatory cytokines by LPS or phytohaemagglutiniin stimulated cultures of whole blood [36] and PBMCs [37] is down-regulated by ethanolic *S. frutescens* extracts. Faleschini *et al*. [38] found that a hot aqueous extract and an ethanolic extract of *S. frutescens*, and fractions of these extracts regulated cytokine expression in PMA-stimulated HL60 cells, with differential regulation of the pro-inflammatory cytokines TNFα and IL8. The ethanolic preparations increased the release of TNFα and IL8, while the hot aqueous extract and fractions decreased the release only of IL8, demonstrating the differential action of the phytocompounds and phytocompound combinations present within these two extracts [38]. Fortuin-Seedat [39] extended these findings to THP-1, a human monocyte cell line that can be stimulated to differentiate into M1 or M2 macrophages depending on the stimulus. Aqueous and ethanolic *S. frutescens* extracts decreased phosphorylation of members of the MAPK, p38 and NFκB signalling pathways and nuclear translocation of the phosphorylated NFκB subunits, p65 and p50, with a consequent reduction in expression of the pro-inflammatory gene products COX2, TNFα, IL-6 and MIP-1β [39]. Regulation of the MAPK pathway occurred by Akt-independent regulation of MAPKs, ERK1/2 and p38 and Akt-dependent (and/or independent) regulation of the NFkB pathway, with possible involvement of the GSK3β and/or the IKK signalling pathways [39].

Thus, therapeutically targeting macrophage-mediated inflammation with *S. frutescens* has potential to treat numerous inflammatory conditions. In addition,

stimulation of M2-mediated wound healing provides an alternative clinical approach.

5. Macrophages from tissue damage to repair

Tissue damage during infection and following toxic or mechanical injury results in the release of damage-associated molecular pattern (DAMP) molecules by dead or dying cells and production of PAMP molecules by invading pathogens [131]. DAMPs and PAMPs induce a complex inflammatory response involving recruitment, proliferation, and activation of a range of cell types, including neutrophils, macrophages, NK cells, B cells, T cells, fibroblasts, epithelial cells and endothelial cells that together produce a response that simultaneously controls inflammation and stimulates tissue repair (also known as wound healing) [132].

Wound healing is a highly flexible process. Macrophages, in particular, play a vital role at all stages of wound healing and fibrosis [92, 133]. However, if the wound healing response is disrupted or becomes chronic, then pathological fibrosis, scaring, organ failure, or death can occur [134]. Therefore, it is critical that the wound healing process is tightly regulated to allow rapid resolution of inflammation and to restore normal tissue structure.

5.1. Macrophage-mediated wound healing

Wound healing mediated by the monocyte/ macrophage lineage involves the progression of M1 to M2c macrophage activation (Fig. 11), along a continuum from early stage pro-inflammatory M1 macrophages to later stage pro-reparative M2 macrophage activation. The characteristics and time course of the healing phases are dependent on the severity of the injury, the organ or tissue injured, the age of the affected individual and the overall health of the individual. In the most commonly injured parts of the human body i.e. the skin and muscle, the inflammatory phase normally lasts 1-2 days, with the proliferation phase peaking approximately 1 week post injury, whilst the remodelling phase can last for months [135-138] (Fig. 11).

During the inflammatory phase (Phase 1) (Fig. 11) a mixed M1 and M2a phenotype response occurs [139]. One of the first cytokines to be secreted is IL-4 [140], which stimulates arginase activity, supporting production of new local extracellular matrix by converting arginine to ornithine, a precursor of the polyamines and collagen found in many extracellular matrices [141]. This early production of IL-4 generates M2a macrophages and stimulates resident macrophages towards wound healing. In the subsequent early proliferative phase (Phase 2) (Fig. 11) both pro-inflammatory cytokines and IL-10 and anti-inflammatory markers are produced as the M2a macrophages develop towards an M2b phenotype [139, 142]. In later proliferative stages the increase in IL-10 stimulates M2b macrophage activation of M2c macrophages, indicated by an increase in the M2c marker, TGF-β [142]. The remodelling phase (Phase 3) (Fig. 11) primarily consists of M2c macrophages, characterised by high TGF-β production and CD206 expression, with a decrease in arginase-1 [135].

Once remodelling is complete, inflammation resolves, and the macrophages adopt a deactivated phenotype. Successful wound repair is thus characterised by a return to basal tissue macrophage populations within weeks of the initial injury, synchronised with wound closure and healing. Chronic wounds do not heal after 3 months and show persistent macrophage activation [143].

5.2. Macrophage disregulation during wound healing in metabolic diseases

A pathological state of inflammation causes delayed, incomplete or poorly coordinated wound healing, with wounds failing to heal. Such wounds develop into chronic ulcers, often found in obese patients and those suffering from ischemia, T2D, venous stasis and pressure ulcers [144].

5.2.1. Obesity, diabetes and wound healing

Obese people suffer from local wound complications, such as skin wound infections, hematoma or seroma, pressure ulcers and venous ulcers [144]. Locally increased tension at the edges of surgical wounds contributes to dehiscence, exposing damaged tissue and increasing the risk of infection. This tension at the wound site also increases pressure within the tissue, reducing fluid perfusion and the availability of oxygen to the wound site [145]. Excess skin in obese individuals leads to the formation of skin folds which provide sites for micro-organisms to multiply and contribute to local infection and tissue destruction [144].

Systemic factors, such as enlargement of adipose tissue, further contribute to impaired wound healing. Adipose tissue secretes adipokines, including adiponectin, resistin and the hormone leptin, that are produced by both adipocytes and macrophages within the tissue [144]. Leptin controls energy metabolism by reducing food intake and body weight [146]. Decreased sensitivity of the leptin receptor (LEPR) in cells of the hypothalamus, adipose, skeletal muscle, and liver results in leptin resistance and further increased circulating levels of leptin [147], characteristic of obesity [148, 149].

Leptin acts as a pro-inflammatory cytokine by up-regulating phagocytic activity in monocytes [150] *via* phospholipase activation [151] and inducing the production of eicosanoids, NO, leukotriene B4, cholesterol acyl-transferase-1 and COX-2 [151-153]. Leptin also stimulates monocyte proliferation *in vitro* with up-regulation of activation markers, IL-2Rα, CD69, CD39, transferrin receptor CD71, and IL-1Rα [153]. Leptin activates IRS-1, PI3K/Akt and NFκB pathways in microglial macrophages to induce IL-6 production and activates STAT3 to induce IL-1β production [154, 155]. Leptin stimulates chemotaxis and activation of neutrophils [156], and protects neutrophils from apoptosis *via* the PI3K and MAPK pathways [157]. Skin wound healing was shown to be delayed in Leptin-deficient ob/ob mice, but administration of Leptin exogenously enhanced regrowth of the epithelial cells in the skin wound, restoring healing and indicating a role for Leptin in the completion of the inflammatory response and tissue repair [158].

Diabetic individuals have impaired acute wound healing, and are susceptible to developing chronic non-healing diabetic foot ulcers (DFUs) [144, 159] and pressure-related chronic non-healing wounds associated with local hypoxia [160]. Prolonged local hypoxia in these complications is caused by insufficient perfusion and insufficient angiogenesis, which exacerbates the early inflammatory response, increases the local levels of oxygen radicals and prolongs the injury [161, 162]. Other abnormalities of diabetic wounds include inadequate T cell immunity, defective leukocyte chemotaxis and phagocytosis and abnormal repair functions in fibroblast and epidermal cells. As a consequence there is poor clearance of local bacterial infections and impaired or delayed tissue repair [163].

Chronic low-grade inflammatory activation is found in patients with abdominal obesity and may be involved in the pathogenesis of obesity-related disorders, including T2D [164]. The progression from IR associated with obesity to T2D reflects the failure of pancreatic β cells to produce sufficient insulin to compensate for the IR. This reduction in insulin results in chronic hyperglycaemia and inflammation of the pancreatic islets, with associated fibrosis and increased β cell death. Consequently there is local infiltration of macrophages and increased pro-inflammatory cytokines and chemokines [68]. The pro-inflammatory cytokine IL-1β is seen within pancreatic islets of T2D individuals [165]. This cytokine is thought to be a key regulator of islet inflammation, acting locally to increase other pro-inflammatory cytokines and chemokines, leading to recruitment of immune cells [166]. Adipokines, such as Leptin, chemokines and fatty acids can also modulate macrophage phenotype and function. Adiponectin promotes a M2-like phenotype in adipose tissue macrophages, helping to maintain adipose tissue homeostasis [167]. In contrast, obesity-associated fatty acids bind to and activate macrophage TLR4, promoting a pro-inflammatory M1 phenotype [168, 169].

The pathology of T2D and diabetic wounds thus reflects a chronic inflammatory state that persists due to imbalances in pro- and anti-inflammatory cytokines, where the M2-dominated repair phase is attenuated. Therefore, T2D progression and diabetic wound healing could be improved by resetting the balance between M1 and M2 macrophage subsets [170]. Such a reset may be possible using topically applied *S. frutescens* extracts to promote wound healing.

6. Conclusion

Commonly used pharmaceutical medications to treat inflammatory disease include anti-coagulants, anti-platelet medications, glucocorticoid steroids, non-steroidal anti-inflammatory drugs (NSAIDs), and some chemotherapeutic drugs [144]. However, such drugs can impair wound healing. Medications that inhibit coagulation and platelet formation potentially inhibit the early stages of wound healing [171] and therefore have limitations in the

treatment of T2D, in which wound healing may be compromised [172]. Glucocorticoids produce beneficial effects in pathophysiological conditions such as rheumatoid arthritis; however, the anti-inflammatory and immunosuppressant effects of these steroids can also delay wound healing [171] and increase the risk of wound infections. Systemic administration of the NSAID ibuprofen reduces cell proliferation in wound healing, resulting in a decrease in fibroblast numbers, limited wound contraction and epithelialization and impaired angiogenesis [144, 173].

Medicinal plant preparations including aloe vera, tea tree oil, echinacea, chamomile, ginseng, mimosa, jojoba, garlic and ginkgo have been used as topically administered treatments, to alleviate inflammation and aid in wound healing [174, 175]. *S. frutescens* shows great potential as a phytomedicine for this field of medicine. Currently there is no single bioactive phytochemical identified in either hot aqueous or ethanolic *S. frutescens* extracts that accounts for their anti-inflammatory activity. We postulate that this anti-inflammatory action may reflect the combined effect of several phytochemicals. Adefuye [16], in a phytochemical analysis of ethanolic and hot aqueous *S. frutescens* extracts, showed that a 100% ethanolic extract had a significantly higher flavonol and tannin content compared to the hot aqueous extract. This may explain the higher activity of the ethanolic extract in inhibiting M1 macrophage function, since flavonol and tannins are polyphenols that have anti-inflammatory activity [176]. It is likely that synergistic or combined action of more than one phytocompound is required for the observed anti-inflammatory action of *S. frutescens*, since, to date, no purified compound has been as effective as the extracts tested, either *in vitro* or *in vivo*. Published work indicates that *S. frutescens* has potential as a phytomedicine for treatment of inflammation and to stimulate wound healing, *via* down-regulation of pro-inflammatory macrophage activity. We propose that this involves decreasing phosphorylation of members of the pro-inflammatory MAPK, p38 and NFκB signalling pathways with consequent decreases in expression of pro-inflammatory genes COX2, TNFα, IL-6 and MIP-1β. Additionally regulation of macrophage apoptosis through ERK1/2 phosphorylation could stimulate recruitment of more phagocytic macrophages to an inflamed area to aid in the resolution of inflammation and wound repair. Inhibition of the Akt-independent IKK pathway and activation of the Akt-dependent GSK3β signalling may involve negative feedback action by GSK3β, which regulates inflammation associated with pathophysiological conditions such as IR, T2D and obesity. The differing findings with aqueous and organic extracts indicate that care is needed in the use of such extracts, in the source of the plant material, the choice of systemic or topical application and the dosage used. We conclude that application of such differing extracts of *S. frutescens* can be tailored either to stimulate the clinically required immune responses or, in other clinical situations, to down regulate harmful inflammatory responses.

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CONFLICT OF INTEREST STATEMENT

The authors state that there are no conflicts of interest in this work.

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