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## REVIEW ARTICLE

# The Modulatory Effect of Lead Drug Candidates on Inflammatory Gene Expression in Sepsis: A Mini-Review

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**Abstract:** Sepsis is a debilitating clinical syndrome of systemic inflammation in response to microorganisms especially Gram-positive and Gram-negative bacteria. A minority of sepsis cases could be due to non-pathogenic insult such as trauma. Much of the tissue and organ injury observed among septic patients is a consequence of the inflammatory response. The search for effective treatments of sepsis has led to several studies by different research groups across the globe. Although many targets and molecules have been identified, there is still no effective treatment for sepsis. The aim of this report is to review the literature on drugs and drug candidates against sepsis and how they modulate the expression of inflammatory genes. Many compounds have been identified to regulate inflammatory gene expression by interacting with targets such as topoisomerase 1 and nuclear factor kappa B, which regulate the production of pro- and anti-inflammatory cytokines. Even though these compounds appear promising as potential drugs against sepsis, no effective therapies have been discovered to date and thus the fight against sepsis continues.

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**Keywords:** Sepsis, Drug candidate, Gene regulation, Inflammation, NF-kappa B.

## 1. INTRODUCTION

Inflammation is a natural process used by the immune system to protect the body from infectious foreign materials such as bacteria and viruses [1]. With a pathogenic insult, the body mounts an immune reaction (the pro-inflammatory phase) producing several cytokines such as interleukins (IL-1, IL-6, IL-8) that activate resting macrophages and lymphocytes to phagocytose and destroy the invading pathogen. To ensure the immune system does not over-respond but keep the response equal

to the strength of the invading pathogen, anti-inflammatory cytokines are also released to more or less suppress the effect of the pro-inflammatory cytokines. For example, IL-1Ra (an anti-inflammatory cytokine) is released to compete with and inhibit the activity of IL-1 [2]. An uncontrolled inflammatory response that results in cytokine burst and subsequent damage of organs is referred to as sepsis. Sepsis is a clinical syndrome mostly associated with fever, edema and respiratory failure while septic shock encompasses progressive failure and severe damage of organs (organ failure), vascular leakage and shock leading to death [1]. In the classical explanation of the mechanism of sepsis, pro-inflammatory responses are considered the major mediators of sepsis.

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The innate immune response is known to be the first response to microbial infection. The innate immune response sets in via the activation of a series of transmembrane and cytosolic Pattern Recognition Receptors (PRRs) which detect Pathogen Associated Molecular

Patterns (PAMPs) or microbe associated molecular patterns (MAMPS), the conserved microbial structures and motifs [3]. The most studied PRRs are the Toll-like receptors (TLRs). It is important to note that regulation of the expression of the PRRs serves as a target for sepsis drug discovery and development.

Macrophages are immune cells that migrate to the site of infection during the first few days of an infection to phagocytose the pathogen. The phagocytes produce pro-inflammatory cytokines such as interleukins - IL1 $\beta$ , IL-8, IL-6 and TNF. Inhibition of the production of these cytokines or their downstream signaling cascades can also serve as a drug target for sepsis. The innate immune cells or infected cells expressing Toll-like receptors (TLRs) sense the molecular motifs specific to pathogens (PAMPs/MAMPS) and send signals via the nuclear factor (NF)- $\kappa$ B [4] or through the JAK-STAT signaling cascades [5]. The TLR – NF-  $\kappa$ B signaling leads to the production of nitric oxide which induces vasodilation and increased blood flow to the site of infection. The signaling also stimulates the production and release of pro-inflammatory cytokines such as TNF, IL-1 $\beta$ , IL-6 and chemokines. The release of the cytokines and chemokines leads to the recruitment of monocytes and neutrophils to the infected site. Secreted cytokines also increase the pro-coagulant properties of endothelial cells and induce platelet-neutrophil interactions which lead to the formation of neutrophil extracellular traps (NETs) [6].

If the immune system is able to inhibit the growth and propagation of the pathogen, then sepsis will not occur; but if the invading pathogens are able to spread outside the site of infection, then the specific host defense mechanisms can shift from beneficial to detrimental. In a septic situation, both the infection and the inflammatory response to the infection becomes systemic, hence resulting in diffuse organ injury and shock [7].

To date, there is no effective approved drug for treating sepsis [8] as clinical trials of drugs to alleviate sepsis have not been successful so far. [9,

10]. In most sepsis drug trials, the small sample size and incomplete understanding of the pathophysiology of sepsis have been noted as the major reasons for the lack of success. One of the therapeutic approaches pursued alleviating sepsis has focused on the modulation of pro- and anti-inflammatory gene expression [11, 12]. The response of the immune system is largely dependent on the expression of specific genes which act directly as immune effectors to activate specific pathways [12]. Therefore, therapies targeting the regulation of gene expression of pro- and anti-inflammatory molecules appear to be promising drug candidates for the treatment of sepsis. This review examines the major pathways that therapies meant to alleviate sepsis target to modulate the expression of pro- and anti-inflammatory genes.

## 2. METHOD

A systematic search was done with the following key terms on PubMed and Google scholar [drug-therapies (AND sepsis, AND pro-inflammatory, AND/OR anti-inflammatory cytokines, AND gene expression)]. The literature search was conducted from April 2017 to February 2018 with 443 search results. The titles of the articles were first screened to ensure that they are original research articles in the subject area. Out of the initial screening, 102 publications were identified and screened further based on the content of their abstracts to obtain 70 articles which were considered for the study.

The inclusion and exclusion criteria below were used to select the research articles used in this review.

### Inclusion criteria

1. The article must be primary literature and should include wet laboratory study
2. The study must be focused on drug discovery in sepsis.
3. The compounds used as drug candidates must influence gene expression of pro- and/or anti-inflammatory cytokines
4. Studies that used both in vitro and in vivo or only in vivo approaches

### Exclusion criteria

1. Articles on sepsis treatment using crude extracts or herbal preparations

2. Studies that used only *in vitro* approaches
3. Studies based on gene therapy

### 3. RESULTS

#### 3.1. Modulating Gene Expression in Sepsis

The innate immune response to bacterial infection in sepsis is largely due to altered expression of more than 3,700 genes [13]. Whole-genome expression profile of whole blood from septic patients have been shown to provide critical biological insights on the expressed genes in septic patients, leading to novel approaches to sepsis diagnosis, prognosis and therapeutic interventions [14]. Statistical analysis of gene expression data is fraught with challenges due to lack of clear classification of differences in expression among patient groups. Nevertheless, RNA sequencing and microarray-based expression studies have provided more insights into the complex pathogen recognition and signaling pathways of inflammation in sepsis [14, 15]. Genes that are involved in immune regulation (positive and negative regulation), mitochondrial functions such as oxidative phosphorylation and ATP synthesis, and others modulating inflammatory responses provide candidate targets for drug discovery efforts for the treatment of sepsis [15].

Drug treatments that inhibit pathways leading to pro-inflammatory gene expression are promising candidates for the development of drugs against sepsis. It was therefore of interest to review some of the promising pathways as well as candidate drugs that modulate inflammatory gene expression.

#### 3.2. Topoisomerase 1 Inhibition

In all cell types, the activity of RNA polymerase II depends on the action of topoisomerase to resolve topological constraints on the DNA [16]. Topoisomerase 1 was found to be redundant in controlling the expression of housekeeping genes but its inhibition suppresses the expression of long genes (>100kb) although it is able to induce just a fraction of smaller genes [16]. The selective suppression of gene expression by topoisomerase 1 probably allowed for the suppression of inflammatory genes induced by microbial pathogens. *In vitro* assays demonstrated that the inhibition of topoisomerase during different bacterial infections

led to the suppression of RNA polymerase II recruitment at PAMP induced sites [17]. The above effect may be explained by either interference of co-transcriptional events such as RNA stability/transport or nucleosome remodeling by topoisomerase 1 inhibition. In a mouse model, inhibition of topoisomerase has proven to be effective in protecting mice from septic shock and death by suppressing both anti-viral and anti-bacterial inflammatory signals induced by the host gene expression. Rialdi *et al.* reported that inhibiting topoisomerase 1 activity with the cancer drug camptothecin (CPT) led to a significant reduction in expression levels of pathogen-associated molecular patterns (PAMP)-induced genes [17]. These PAMP-induced genes were type 1 interferons (IFN) and other cytokines such as interferon-induced protein with tetrapeptide repeats (IFIT1 & IFIT2) which when expressed bind to immune cells with the corresponding receptors activating them and exacerbating the immune response further. Thus, inhibiting the expression of such genes reduced the exaggerated immune response. The hypothesis was confirmed by infecting mice with the deadly Influenza and Ebola viruses and blocking the activity of topoisomerase 1 in their treatment cohorts using a synthetic drug [17]. The exaggerated expressions of antimicrobial or antiviral genes have been linked to the high mortality rates experienced in sepsis. Rialdi and colleagues reported significant survival rates in their treatment groups while almost all the animals in the control group without the topoisomerase-inhibitor died (as a result of the exaggerated immune response) [17]. This suggests a gene specific activation role for topoisomerase 1 in regulating sepsis. Although the cellular response of the mammalian immune system against infection is needed for protection against the infectious pathogens, hyper activation can have deleterious effects on man. These adverse effects of the over-exaggerated immune response which causes sepsis and septic shock can be controlled to a large extent by inhibitors of topoisomerase 1 such as Flavopiridol and Camptothecin [17].

#### 3.3. Nuclear Factor- $\kappa$ B (NF- $\kappa$ B) Repression

Pro-inflammatory cytokines are positively regulated by nuclear factor  $\kappa$ B (NF- $\kappa$ B), an inducible transcription factor. NF- $\kappa$ B is ubiquitously made in almost every cell and bound to its inhibitory molecule I $\kappa$ B in the cytosol. Upon activation, NF-

$\kappa$ B is released from I $\kappa$ B and relocates to the nucleus to bind promoter sequences of target genes [18]. However, the availability and activation of NF- $\kappa$ B depending on host epigenetic mechanisms [18]. Binding of inhibitory molecules to NF- $\kappa$ B receptors have the potential to repress the transactivation of NF- $\kappa$ B. Repression of the expression of NF- $\kappa$ B subsequently down-regulates the activity of pro-inflammatory cytokines [19, 20]. Considering the role of NF- $\kappa$ B in regulating the repression of pro-inflammatory cytokines, drugs aimed at suppressing the production of NF- $\kappa$ B are potential anti-sepsis drug.

Esculin, a compound obtained from Chinese medicinal herbs, has proven to be a promising drug candidate for the treatment of sepsis. The herb was traditionally used as an anti-inflammatory drug in China. It has been demonstrated that esculin suppresses inflammatory reactions in macrophages and protected mice from LPS-induced endotoxin septic shock [1]. In a survival assay using mice, esculin pretreatment significantly improved the survival rate of LPS-induced mice. It was proposed that the induction of the anti-inflammatory response protected the LPS-induced sepsis mice from lungs, liver and kidney tissue damage. To confirm the above claim, the expression levels of NF- $\kappa$ B subunit p65 in lung, liver and kidney were studied. Treatment with esculin significantly suppressed protein expression of NF- $\kappa$ B in these organs suggesting that esculin is a potential drug for treatment of inflammatory diseases including sepsis [1]. Even though the mechanism of Esculin, is not well known, it has been reported that nitric oxide (NO) production is significantly inhibited by this compound through the inhibition of nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation in macrophages (add reference).

Another compound,  $\gamma$ -amyrone, obtained from *Sedum lineare*, a plant used in traditional medicine for its anti-inflammatory activity also reduced the production of NF- $\kappa$ B. The study results indicated that  $\gamma$ -amyrone treatment significantly decreased mortality rate, due to the reduced p65 subunit of NF- $\kappa$ B protein expression in LPS-induced sepsis mice models compared with the control group [21]. Application of  $\gamma$ -amyrone protected the liver and kidneys of the animals by decreasing the accumulation of nitric oxide in the serum. It has been suggested that the protective effect of  $\gamma$ -amyrone on LPS-induced endotoxin shock may be due to

the suppression of p65 NF- $\kappa$ B protein and nitric oxide levels which mediates the production of pro-inflammatory cytokines [21].

Paeoniflori, a Chinese compound also prevented the activation of NF- $\kappa$ B and subsequently increases the survival of LPS-induced sepsis mice [22]. In a similar study, corylin, a medicinal plant product was found to reduce the expression of NF- $\kappa$ B in both LPS-activated RAW 264.7 cells and LPS-activated murine peritoneal macrophages [23]. Trilinolein is a natural product from *Panax notoginseng*. In a study using LPS-stimulation in mouse macrophages to induce sepsis, the role of trilinolein in preventing sepsis was examined. The study results suggested that trilinolein suppressed the protein expression of NF- $\kappa$ B, I $\kappa$ B $\alpha$ , and mitogen-activated protein kinases (MAPKs) [24]. Summarized in Table 1 are other compounds found to be repressors of NF- $\kappa$ B and affecting the differential expression of inflammatory genes.

Associated complications of sepsis include coagulopathy, endothelial dysfunction and cell death (apoptosis and necrosis). Modulating gene expression levels of the antithrombotic protein C (APC) altered the expression of NF- $\kappa$ B. With this knowledge, a synthesized recombinant active form of APC (rhAPC) experimentally reduced the expression of the individual subunits of NF- $\kappa$ B (p50 and p52) that essentially reduced the expression of TNF- $\alpha$ -induced genes and other pro-inflammatory cytokines in sepsis pathogenesis [25].

### 3.4. Inhibition of Pro-Inflammatory Cytokines and Activation of Anti-Inflammatory Cytokines

The Chinese medicine, paeoniflori from paeony root was shown to have anti-inflammatory activity against LPS-induced cardiac dysfunction in mice [22]. The administration of paeoniflori significantly reduced the expression of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1b, IL-6, IL-12, MCP-1, and IFN-g. Paeoniflori administration inhibited the production of inducible nitric oxide synthase (iNOS) and thus increasing the survival of LPS-induced septic mice models. In a similar study, esculentic acid, a completely different compound to esculin from Chinese medicinal plant was found to decrease the levels of TNF- $\alpha$ , IL-6, COX-2 protein expression and NO production that subsequently increased the survival of mice but with LPS-induced endotoxic shock [26]. In addition to the compounds mentioned above, corylin

[23],  $\beta$ -thujaplicin [28], nodakenin [29] and trilinolein [24] were also found to inhibit the production of TNF- $\alpha$ , IL-6 and NO in LPS-induced sepsis mice models. It is worth mentioning that esculentin acid and esculin (discussed earlier) are completely different molecules.

Hydrostatin-TL1 is a collection of peptides in the venom of a sea snake. The peptide collections were found to have anti-inflammatory activity by reducing the levels of TNF- $\alpha$ . The venom prevented the interaction between TNF- $\alpha$  and its receptor and reducing the cytotoxicity of TNF- $\alpha$  in L929 cell lines. The venom peptides demonstrated high level of protection and increased survival of mice with dextran sodium sulfate (DSS)-induced acute colitis and lipopolysaccharide (LPS)-induced acute shock [27].

Kukoamine B (KB) is another promising anti-sepsis drug [28, 29] that inhibits the over expression of pro-inflammatory cytokines induced by CpG DNA. Mice challenged with heat-killed *Escherichia coli* were protected with KB by selectively inhibiting LPS- and CpG DNA-induced pro-inflammatory pathways without interfering with cell viability pathways in macrophages [29]. KB was also found to trigger a conformational change in CpG DNA upon binding to it on the surface of a biosensor chip [28].

### 3.5. Ubiquitin-Proteasome Pathway Inhibition

In the protein degradation pathway, ubiquitination of proteins that regulates cellular activities such as inflammatory processes, cell cycle regulation, and gene expression leads to their degradation [30]. The cell-permeable proteasome inhibitor, MG-132, significantly reduced the plasma levels of TNF- $\alpha$ , IL-1, IL-6 and IL-10 after a caecal ligation and puncture (CLP) to induce sepsis. MG-132 treatment prolonged survival of CLP-induced sepsis mice by decreasing the inflammatory responses through the ubiquitin-proteasome pathway. Velcade<sup>®</sup> (Bortezomib) was found to be involved in proteasome inhibition and serving as a potential treatment option for cancer and inflammatory conditions by suppressing cytokine storm [31].

### 3.6. Inhibitors of Macrophage Migration Inhibitory Factor (MIF)

Macrophage migration inhibitory factor (MIF) also known as glycosylation-inhibition factor (GIF) is a major regulator of the innate immune

system and involved in regulation of cell mediated immunity and inflammation [32]. MIF has been connected to conditions such as sepsis, inflammation and autoimmune disease illuminating the role of MIF as a potential target for sepsis drug discovery efforts [33, 34]. In one experiment, phenolic hydrazones were identified as inhibitors of MIF and protected against sepsis [35]. The administration of these compounds suppressed TNF- $\alpha$  secretion from LPS-stimulated macrophages [35].

### 3.7. Inhibitors of Signal Transduction Pathways

The protein expression of mitogen-activated protein kinases (MAPKs) and other signal transduction mediators including NF- $\kappa$ B were inhibited by trilinolein in a study by Huang and his coworkers [24]. Their results implicate the RAS/MAPK pathway in regulating the expression of inflammatory genes in the nucleus of the immune cells. Among the immune regulatory proteins is the mysterious cytokine, MIF, which plays an important role in regulating several immune response pathways in the cell including the RAS/MAPK pathway (Fig. 1) [35, 36].

### 3.8. Other Drugs with Sepsis Indication but does not Target Inflammatory Gene Expression

Over the past 40 years, a number of studies have been conducted to find anti-sepsis drugs after the failure of J5 antisera and R595 at clinical trial levels [37, 38]. In this section, we focus on the clinical trials of drugs that do not target inflammatory gene expressions but have sepsis indications.

Corticosteroids have been reported to inhibit the production of key cytokines such as TNF and IL-1. They were also found to be effective in inhibiting nitric oxide synthase as well as decrease the release of platelet-activating factor. Corticosteroids were therefore expected to be effective in treating sepsis [39]. This hypothesis was tested in clinical studies with sepsis patients and recorded reduced mortality with the administration of methyl-prednisolone or dexamethasone. However, administration of high doses of these drugs did not give any benefit on patient outcome [40-42]. In a similar pattern, continuous infusion of hydrocortisone did not lower mortality compared to the placebo [43].

Aspirin has been implicated to play a role in the activation of platelets in sepsis [44]. The U.S. Na-

tional Library of Medicine in a clinical trial, investigated the effectiveness of aspirin to reduce organ dysfunction in severe sepsis [45]. Based on the results from observational studies, aspirin was found to decrease morbidity and mortality associated with sepsis, however, randomized controlled clinical trials are needed to elucidate the exact role of aspirin in the treatment or prevention of sepsis.

The recombinant form of activated protein C; activated drotrecogin Alpha (DrotAA), has been approved by the FDA as it is known to reduce mortality when administered to patients with severe sepsis and are at high risk of death [46]. However, the challenge with DrotAA administration is patient selection as it is not indicated for use in sepsis patients with low death risk and may also induce slight bleeding tendencies [46].

### 3.9. Challenges in Sepsis Drug Discovery

Understanding the natural history of a medical condition usually precedes large-scale drug discovery and development projects [11, 12]. In sepsis, however, there is limited knowledge of the underlying biology of the disease making it difficult for clinical trials to be undertaken. The few trials completed over the recent years have also yielded inconsistent results, where the cytokine responses in humans with the infection varied from infection to infection and from study to study [12].

The majority of sepsis studies were carried out in cell lines and animal models where the results are not necessarily directly transferable to humans. Another major challenge to undertaking human studies is the difficulty for the patients and their healthcare providers to detect the onset of the disease since it usually starts as a sub-acute illness [11]. In most clinical trials, sepsis drugs usually fail at phase II or III due to the inconsistency of the cytokine responses observed in patients [11, 27, 47] coupled with the few number of participants in the trial [11].

In most preclinical studies LPS was used to induce sepsis in mice, which does not mimic complete pathogenesis of the condition [22, 23, 27]. Cecal ligation and puncture in animal models seem to be a better replica of the natural pathogenesis of sepsis in humans. The results of pro-drugs screened in this kind of experiment may not be comparable to the natural cases.

**Table 1. Compounds that target inflammatory gene expression pathways in sepsis treatment.**

	Compound	Source	Mode of action	Reference
1	Flavopiridol Camptothecin	Synthetic flavonoid <i>Camptotheca acuminata</i>	A and F	[17]
2	Esculin	<i>Cortex Fraxini</i>	B and C	[1]
3	$\gamma$ -Amyrone	<i>Sedu lineare</i>	B and C	[21]
4	Paeoniflori	Chinese medicine	B and C	[22]
5	Esculentic acid	<i>Phytolacca esculenta</i>	C	[26]
6	Hydrostatin-TL1	<i>Hydrophis cyanocinctus</i>	B	[48]
7	PS-341	Synthetic	D	[30]
8	MG-132	Synthetic	D	[49]
9	Corylin	<i>Psoralea corylifolia</i>	B and C	[23]
10	$\beta$ -thujaplicin	<i>Chamaecyparis obtusa</i>	B and C	[50]
11	Nodakenin	<i>Angelicae gigas</i>	B and C	[51]
12	salicylates and glucocorticoids	Plant sources	B and C	[52]
13	Mixture of compounds	<i>Andrographis paniculata</i>	B	[53]
14	Trilinolein	<i>Panax notoginseng</i>	B, C and E	[24]
15	Kukoamine B	<i>Lycium chinense</i>	C	[28]

A=Topoisomerase 1 inhibitors; B=Repressors of nuclear factor kappa  $\beta$ ; C=Inhibitors of pro-inflammatory cytokine production; D=Inhibitors of ubiquitin-proteasome pathway; E=Inhibitors of signal transduction pathways; F=cyclin-dependent kinase (cdk) inhibitor

## 4. DISCUSSION

Regulation of the levels of pro- and anti-inflammatory cytokines has emerged as a promising approach to the effective treatment of sepsis. One of the approaches to regulate the expression of inflammatory cytokines (such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, MCP-1, and IFN- $\gamma$ .) is through the selective inhibition of transcription of proteins involved in the synthesis of these molecules. Topoi-

somerase 1 inhibition has been found to selectively inhibit the expression of inflammatory genes with no or minimal effect on housekeeping genes in LPS-induced sepsis model [17]. The promoters of RNA transcription factor II are modulated by topoisomerase 1 in such a fashion as to regulate PAMP-induced gene expression (Fig. 1). Since topoisomerase 1 inhibitors protect against lethal inflammation, they serve as an attractive drug candidate class for the development of sepsis treatments.

A protein encoded by the RAF1 (v-raf-1 murine leukaemia viral oncogene homolog 1) gene plays a critical role in regulating the mitogen-activated protein kinase pathway in the inflammatory response. Expression of this gene is reduced during the inflammatory response in septic patients suggesting infection may have suppressed the neutrophil's natural inflammatory response to pathogens [15, 54].

During exacerbated inflammatory reaction in sepsis, inhibition of mitochondrial function is an adaptive host response to preserve cellular integrity and enhance subsequent recovery [55, 56]. This inhibition is significantly less in septic patients, an evidence that adaptive immune response in neutrophils may be impaired in the presence of infection in sepsis pathogenesis [15, 57].

High mortality rates and worse clinical outcome of sepsis are strongly associated with the accumulation NF- $\kappa$ B in the nucleus [58]. During the development of sepsis-induced organ failure, NF- $\kappa$ B is involved in the regulation of several immunomodulatory mediators. Studies have shown that the inhibition of NF- $\kappa$ B restores neutrophil apoptosis to baseline levels. The neutrophil apoptosis restoration reduces acute inflammatory processes and organ dysfunction hence mediating the recovery from sepsis [59]. This review has identified a number of compounds for the repression of NF- $\kappa$ B (Fig. 1). Most of these compounds are components of traditional Chinese medicines (TCM). These compounds alter gene expression to reduce the synthesis of NF- $\kappa$ B proteins and possibly serve as leads in sepsis drug discovery.

In protecting the body against pathogens, the exacerbated pro-inflammatory response triggers pain and deterioration of organs leading to eventual organ damage [17]. In the search for adequate chemotherapy for sepsis, some compounds have

been found to be active against the inflammatory activity of pro-inflammatory cytokines while other compounds interfere with the role of the pro-inflammatory cytokines by reducing their levels in circulation.

In the discovery of anti-sepsis drugs, the proteasome pathway remains less exploited compared to the other targets. The proteasomes are present in all cells as macromolecular complexes with enzymes and scaffolding proteins responsible for the degradation of a wide variety of intracellular proteins [60]. This pathway is actively involved in regulating inflammatory processes by regulating gene expression at the protein level. Agents that inhibit the proteasome have been shown to be active or effective in numerous animal models of inflammation and cancer [Ross-Ellit]. This indicates that the proteasome pathway is a likely target for future anti-sepsis drug discovery and development efforts.

#### 4.1. Way Forward to Finding New and Effective Anti-Sepsis Drugs

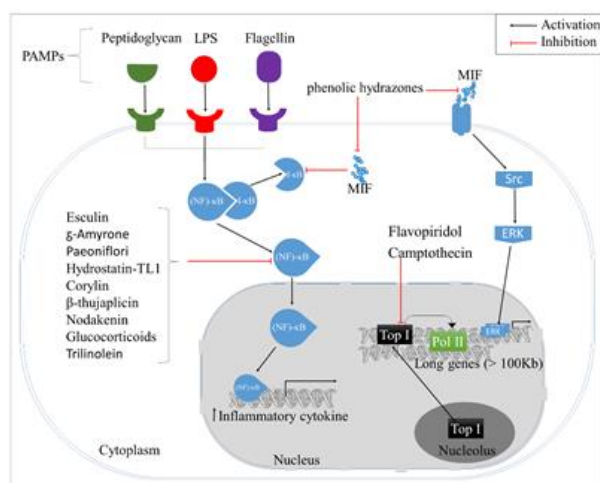
##### 4.1.1. *Insilco and In Vivo Screening of Libraries Using Inflammatory Gene Expression Pathways as Drug Targets*

Target selection and validation is one of the important steps in drug discovery and development [61]. The following inflammatory gene expression pathways were identified as potential drug targets in our report:

1. Topoisomerase 1 inhibition
2. Nuclear factor- $\kappa$ B (NF- $\kappa$ B) repression
3. Inhibition of pro-inflammatory cytokines and activation of anti-inflammatory cytokines
4. Ubiquitin-proteasome pathway inhibition
5. Inhibitors of macrophage migration inhibitory factor
6. Inhibitors of signal transduction pathways (RAS/MAPK pathway)

In sepsis drug discovery, the identified pathways in this study could serve as drug targets for screening chemical and natural libraries to identify new antiseptic agents. We also propose a computational-based study to screen libraries

against some of the targets that modulate inflammatory gene expression in sepsis.



**Fig. (1).** A simplified illustration of inflammation antagonism by drug candidates against sepsis. Binding of bacterial PAMPs to receptors activates the cellular immune response. NF- $\kappa$ B is naturally inhibited by I $\kappa$ B in the cytoplasm, activated NF- $\kappa$ B dislodges from its inhibitor and transported into the nucleus stimulating transcription of inflammatory cytokines. Effects of NF- $\kappa$ B are interfered by a number of sepsis drug candidates (Esculin, corylin etc). Topoisomerase I (Top1) is involved in the transcription of long genes (>100kb). Inhibiting TOP1 decreases transcription of inflammatory-related genes.

#### 4.1.2 Repositioning of Promising Drugs as Anti-Sepsis Agents

The traditional drug discovery process is not sufficient to satisfy the unmet needs in sepsis therapy [62]. To accelerate anti-sepsis drug discovery process and reduce the cost involved, there is a need to reposition some of the known drugs for sepsis therapy. This strategy will also reduce the risk associated with sepsis drugs failing at the clinical stages of the drug development process [63]. It is important to carefully consider drugs against other diseases that may target some of the pathways involved in sepsis pathogenesis. Hall *et al.* 2014, interrogated 1280 approved drugs of which they identified the 10 most potent drugs with anti-inflammatory activities (Appendix Table 1) [62].

#### Anti-Inflammatory Drugs

Some lipids such as *lipoxins*, *resolvins*, *protectins* and *maresin* were known to act as media-

tors to resolve inflammation [64]. They promote the resolution of inflammation through several receptor mediated signaling pathways. This class of drugs might serve as a potential source of anti-sepsis drugs because they have been effective in resolving inflammation in the following conditions; dry eye, retinal disease, asthma, inflammatory bowel diseases, rheumatic arthritis and cardiovascular diseases [64]. In a study by Spite *et al.* 2009, *Resolvin D2*, was implicated as a potent leukocyte regulator to control microbial sepsis [65]. *Resolvin D1* was also identified to improve survival by reducing bacterial load and subsequently preventing the overactivation of the inflammatory response in sepsis-induced mice [66].

#### Analgesic Drugs

Combination therapy or modification of the dosage and/or structure of anti-inflammatory analgesics can make them act as possible anti-sepsis drugs. Aspirin, a common analgesic was found to modulate multiple organ dysfunction in sepsis [44]. The non-opioid, non-steroidal analgesic; *Nefopam*, known to act through several pathways including inhibiting sodium and calcium channels to relieve pain could be a promising anti-sepsis drug [67] as calcium and sodium dysregulation has been implicated in the development of sepsis-induced cardiomyopathy [68] and neuropathy [69]. Appendix Table 1 summarizes the potential drugs for repositioning in sepsis.

#### Anti-Cancer Drugs

Necroptosis plays a major role in the pathophysiology of many inflammatory diseases including sepsis [70]. *Sorafenib Tosylate*, a known drug against cancer [71] can be repositioned as an anti-sepsis drug. The FDA approved drug acts on the RAF/MEK/ERK signaling pathway to regulate gene expression and subsequently inhibit TNF-dependent necroptosis [72].

## 5. CONCLUSION

Different gene regulation pathways modulate the function of the immune system during sepsis and these pathways are the most attractive for the discovery of synthetic and natural products as possible therapeutics for sepsis. In light of lead compounds identified by different researchers targeting these pathways, the potential for successful drug

discovery for sepsis is promising. The majority of these compounds were derived from anti-inflammatory herbal medicines used in their crude form. The compounds reviewed in this study modulated inflammatory gene expression by one or more of the following ways: topoisomerase 1 inhibition, repression of nuclear factor kappa B, inhibition of cytokine production, MIF and inhibition of ubiquitin-proteasome pathway. These are promising therapeutic targets, which need to be further explored, for the discovery and development of effective therapies for the treatment of sepsis.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

### HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

### CONSENT FOR PUBLICATION

Not applicable.

### CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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### APPENDIX

**Table S1. Potential drugs that can be reposition for sepsis.**

Drug	Current use (indication)	Possible target in sepsis	References
Sorafenib tosylate	Anti-cancer	Protein Kinase Inhibitors (RAF/MEK/ERK signaling pathway)	[72]
Nefopam	Analgesic	Calcium and sodium ion regulation	[67]
Aspirin	Analgesic	Modulate multiple organ dysfunction in sepsis	[44]
Lipoxins, Resolvins, Protectins Maresin	Mediators in Inflammation	Reducing excessive neutrophil trafficking to inflammatory loci, Direct regulation of leukocyte adhesion receptor expression Nitric oxide production	[64-66]
Methylthiouracil	Anti-hyperthyroidism	Inhibition of high mobility group box 1 (HMGB1) Restoration of endothelial integrity	[73]
Epinephrine, Norepinephrine, Phenylephrine	Vasopressors and inotropes	Regulate moderate beta-2, strong beta-1 and alpha adrenergic receptor signaling	[74]
Dabrafenib	Metastatic melanoma therapy	B-Raf inhibitor (RAF/MEK/ERK signaling pathway)	[75]

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