Deepti Katiyar1,*, Priya Bansal2, Abhishek Kumar2 and Surya Prakash3

1Department of Pharmacognosy, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad, Uttar Pradesh, India; 2Department of Pharmacology, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad, Uttar Pradesh, India; 3Department of Pharmaceutical Chemistry, KIET School of Pharmacy, KIET Group of Institutions, Delhi-NCR, Ghaziabad, Uttar Pradesh, India

Abstract: Background: Ramalin (γ-glutamyl-N'-(2-hydroxyphenyl) hydrazide), a nitrogen-containing lichen secondary metabolite, was isolated from Ramalina terebrata, an Antarctic lichen. Since then, it has attracted several researchers, thus leading to various research investigations exploring the pharmacological potential of Ramalin.

Methods: The bibliographic databases were explored for the peer-reviewed research related to the pharmacological importance of Ramalin.

Results: The article summarizes the antioxidant, anti-cancer, anti-obesity, antibacterial, and anti-allergic activities of this molecule. Additionally, the studies conducted to show the potential of Ramalin in atherosclerosis, atopic dermatitis, neurodegenerative disorders, hepatic fibrosis and its role in autophagy suppression and enzyme inhibition are also described briefly. Moreover, the experimental findings also depict that Ramalin did not show any toxicity.

Conclusion: The current review shall be beneficial for future researchers interested in working on Ramalin because it summarizes all the relevant publications starting from its first-time isolation to the articles of 2021.

Keywords: Lichen, Ramalina terebrata, ramalin, antioxidant, anti-cancer, anti-inflammatory.

1. INTRODUCTION

Lichens, also known as lichenized fungi, represent a symbiotic relationship between a mycorrhizal fungus (mycobiont) and cyanobacteria or an alga (photobiont). Only 20% of lichens (around 13,500 species and 600 genera) were explored until 1998 [1-3]. They can survive in lingering darkness, drought, constant light, and even in shallow temperatures of -129°F persisting in Antarctica [4, 5], due to which they possess a broad spectrum of secondary metabolites [6-10], which are beneficial for a range of therapeutic activities. Thus, the current review summarizes all the biological activities and possible mechanisms of Ramalin (lichen secondary metabolite) studied until now.

2. RAMALIN

Ramalin was isolated for the first time from the aqueous methanolic extract of Ramalina terebrata, Antarctic lichen [11]. Ramalin is an L-glutamic acid derivative of phenylhydrazide (glutamyl-N’-(2-hydroxyphenyl) hydrazide (Fig. 1), with the molecular formula C11H15N3O4. It was isolated as an amorphous powder (mp, 136.64) [12]. Several investigations have reported the pharmacological activities of Ramalin (Fig. 2), such as enzyme inhibition, antioxidant, antibacterial, anti-allergic, antiatherosclerotic, autophagy suppression, antiatopic dermatitis, anti-cancer, anti-obesity, neuroprotective, anti-inflammatory and anti-hepatic fibrosis and its multi-mechanistic approach in the treatment of various diseases.

3. PHARMACOLOGICAL ACTIVITIES OF RAMALIN AND ITS ROLE IN PREVENTION AND TREATMENT OF DISEASES

3.1. Antioxidant

The disturbed balance between the manufacture and elimination of the reactive oxygen species leads to oxidative stress, resulting in various chronic disorders such as diabetes, cancer, and cardiovascular and neurodegenerative diseases [4, 13, 14]. Thus, a study was conducted to study the HO* and HOO* radical scavenging activity of Ramalin using the aqueous and the lipid media (pentyl ethanoate) environment for in silico assessment through kinetic calculations. Ramalin was found to show 24 times quicker HOO* radical scavenging activity and four times faster HO* radical scavenging potential in polar as compared to nonpolar media. Mainly, the FHT (Formal Hydrogen Transfer) mechanism was the major route through which cleared off the HOO* free radi-
cals in the aqueous as well as the lipid environment resulting in 90% and 30% in the overall HO• radical scavenging, respectively, in the polar and nonpolar medium. Ramalin also exhibited 1.72 times faster HOO• free radical scavenging potential in water and 1.85 times quicker in a pentyl ethanoate environment than Trolox [15].

The study suggested that Ramalin shows an excellent antioxidant action devoid of toxicity, which may be due to its possession of a strong ability to give away electrons and hydrogen. The phenolic, carboxyl, and hydroxyl groups present in Ramalin can donate hydrogen for scavenging the free radicals. Similarly, the presence of three amino groups is responsible for imparting their stability by donating their free electrons to the reactive ions. The existence of multiple hydrogens and electron-donating functional moieties in a solitary compound is accountable for its potent antioxidant activity [14].

In another experiment, Brewer et al. analyzed the antioxidant potential of methanolic aqueous (90:10 v/v) extracts of five polar lichen species, including Ramalina terebrata. The results displayed that around 33–50% of the main compounds of the extracts under study were effective antioxidants [16].

3.2. Anti-cancer

Although numerous developments are taking place concerning techniques for diagnosis, therapy, and prevention of cancer, the number of cancer patients is continuously increasing [17, 18], and it is expected that by 2040 the figure may reach 29.5 million [19]. Suh et al., to assess the anticancer potential of Ramalin in the human colorectal cancer cell line HCT116, conducted a study. Ramalin remarkably suppressed proliferation, induced apoptosis, and exhibited anticancer action based on its concentration. The investigational molecule could also seize the G2/M phase transition of the cell cycle, such as TP53 (tumor protein p53), CDK1 (cyclin-dependent kinase 1), CDKN1A (cyclin-dependent kinase inhibitor 1A), and CCNB1 (cyclin B1). Further, the authors found that Ramalin could gradually cause the elevation in the expression of TP53 and CDKN1A while decreasing the expression of CDK1 and CCNB1 in a dose-dependent manner. Collectively, the results indicated the therapeutic potency of Ramalin in colorectal cancer [20].

Another research investigation carried out by Lee et al. studied the capacity of Ramalin to provoke apoptosis and its mode of action in MCF-7 and MDA-MB-231 human breast cancer cell lines. It was found that the compound could inhibit the development of cells and induce apoptosis based on its concentration. Through stimulation of Bax and suppression of the response of Bcl-2, apoptosis-induced factors and cytochrome c were released, which could thus activate the mitochondrial apoptotic pathway. Additionally, in both types of investigational cell lines, stimulated caspase-8 and caspase-9 were identified, while in MDA-MB-231 cells, only caspase-3 was detected. It has also observed the enhanced level of p62 and LC3-II. Furthermore, 3-methyladenine inhibited autophagy which elevated apoptosis induced by Ramalin and a decrement in Bcl-2 levels and an increment in Bax levels. The study’s findings indicated that autophagy might be a defensive mechanism in opposition to apoptosis in cancer cells treated with Ramalin [21].

The findings of western blotting and MTT assay in another research exhibited that Ramalin reduced the cell multiplicity, induced apoptosis-associated proteins (Bax, BID, AIF), and triggered caspase-3 & 8, respectively. Additionally, an investigation of Annexin V/PI showed a significantly enhanced number of apoptotic cells in MCF-7 cells exposed to Ramalin. Moreover, the molecule could also increase the phosphorylation of ERK1/2, JNK, and p38 based on concentration. Thus, Ramalin can be a potent therapeutic agent against breast cancer [22].

3.3. Anti-obesity

Globally, obesity is one of the major threats to human health, leading to a number of metabolic ailments like cardiovascular diseases, hypertension, diabetes, hepatic steatosis, and osteoarthritis. A research investigation by Kim et al. aimed to evaluate Ramalin for anti-obesity potential and its mechanism in 3T3-L1 preadipocytes and HFD (high-fat diet)-induced obesity in mice. Ramalin in the concentration of
1 mg/ml, 5 mg/ml and 10 mg/ml was utilized to treat 3T3-L1 cells. Ramalin also hampered the maturation and specialization of 3T3-L1 adipocytes by obstructing the adipogenic gene expression together with peroxisome proliferator-activated receptors γ (PPAR γ), CCAAT enhancer-binding proteins (C/EBPs), adipocyte fatty acid-binding protein (aP2) and leptin. The adipogenesis repression interceded via suppression of MAPK Pathways. In addition, Ramalin could also decrease the liberation of IL-6 and TNF-α in 3T3-L1 adipocytes. When 50mg/kg and 100mg/kg dose of Ramalin was administered orally to HFD-fed mice, it was found that the body weight gain decreased along with the abdominal fat accumulation while the intake of food remained the same. Moreover, it also reduced the weight of the organ and basal serum level by hindering liver X receptors (LXRs), lipoprotein lipase (LPL) mRNA expression, and sterol regulatory element-binding protein-1c (SREBP-1c) in HFD-fed mice. The current study thus concluded about the anti-obesity activity of Ramalin along with its mechanism of action [23].

Experimental research by Lee et al. aimed to study the consequence of Ramalin on the maturation and specialization of adipocytes in 3T3-L1 cells. It was seen that when Ramalin was used in non-toxic doses, it was able to decrease the buildup of lipid globules tainted with Oil red O. The Western Blot Assay displayed that Ramalin significantly hindered the magnitude of PPAR γ (peroxisome proliferator-activated receptor γ), c/EBP β (CCAAT/enhancer-binding protein β) and c/EBP α (CCAAT/enhancer-binding protein α) in a concentration-dependent way. Moreover, the real-time PCR assay showed that Ramalin could appreciably reduce the expression of MDI-induced-adipogenesis-related genes (GLUT4, aP2, leptin). Additionally, the phosphorylation of ERK1/2, JNK, and p38 was remarkably reduced in a concentration-dependent way. Furthermore, the results of ELISA exhibited the potential of Ramalin to decrease the production of IL-6 and TNF-α. The investigators thus concluded that Ramalin could be a new effective drug molecule for regulating the genesis of adipocytes in preadipocytes [24].

Further, a study was conducted by Park et al. which aimed to evaluate the anti-obesity action of 50 mg/kg and 100 mg/kg oral dose of Ramalin for eight weeks in thirty-five days old mice kept on a High Fat Diet (HFD). The results exhibited a 25% decrement in body weight gain and significant inhibition in epidymal fat-pad weights in contrast to HFD-fed mice. Moreover, it could also lower the glucose level, total serum level, hepatic triglycerides, HDL (high-density lipoprotein), and LDL (low-density lipoprotein) cholesterol. The findings of ELISA showed that the compound under investigation could significantly hinder the HFD-induced leptin cytokine level. Additionally, a Real-Time PCR assay revealed that adipogenesis markers (HFD-induced adipose tissue genes) were remarkably repressed by Ramalin. This study also supported the potential of Ramalin to control obesity and its associated disorders [25].

3.4. Antibacterial

Paudel et al. conducted experimental research with the aim of assessing the antibacterial potential of compounds isolated from the methanolic extract of Ramalina terebrata. In this study, five secondary metabolites named usnic acid, usminine A, usminine B, usminine C, and Ramalin were isolated through bioactivity-guided fractionation and numerous chromatographic techniques. The qualitative assessment of the antibacterial potential of the methanolic extract and the isolated molecules was conducted through the disk diffusion method, whereas the quantitative evaluation was carried out using the MIC (minimum inhibitory assay). All the separated molecules exhibited their antibacterial action and displayed their response against Staphylococcus aureus and Bacillus subtilis [26].

3.5. Anti-inflammatory

A potent anti-inflammatory agent may aim toward inhibiting the production of nitric oxide (an inflammatory mediator) in macrophages. Consequently, a study was conducted to show that Ramalin could inhibit the secretion of inflammatory mediators in macrophages such as the Raw 264.7 cell line. The findings of the RT-PCR and western blot evaluation showed that the investigational molecule could down-regulate the mRNA and protein levels of iNOS resulting in suppression in the level of release of nitric oxide. The observations of the western blot analysis also displayed that Ramalin revoked the phosphorylation of p38, ERK, and JNK induced by lipopolysaccharide along with the abrogation of p65 expression. Luciferase promoter assay also confirmed that Ramalin could attenuate the action of NF-κB. Finally, Ramalin could reduce the release of nitric oxide in the macrophage via down-regulating the signaling of TLR4, which is responsible for suppressing the action of MAPK and NF-κB. Thus, Ramalin can be a potent candidate against inflammation [27].

Park et al. carried out another research investigation to study the consequence of Ramalin on the expression of VCAM-1 (vascular cell adhesion molecule-1) initiated by TNF-α in vascular smooth muscle cells (VSMCs). Ramalin was found to inhibit TNF-α-induced VCAM-1 expression based on its concentration when VSMCs were pretreated with the investigational compound at 0.1-10 μg/mL. The prior treatment with Ramalin remarkably decreased the intracellular free calcium ion level, which TNF-α increased. Moreover, it also hindered human acute monocytic leukemia cell line (THP-1) cell adhesion to TNF-α-stimulated VSMCs. Additionally, Ramalin repressed PADI4 expression, generation of reactive oxygen species (ROS), and phosphorylation of p38, ERK, and JNK. Ramalin also inhibited the TNF-α-induced transfer of NF-κB and AP-1. Thus, the investigation provided the mode of action of Ramalin responsible for altering inflammatory diseases such as atherosclerosis [28].

3.6. Anti-allergic

Jang et al. planned to assess the anti-allergic potential of Ramalin on TNF-α stimulated HaCaT (Human keratinocyte) and RBL-2H3 (antigen mediated rat basophilic leukemia) cells. The findings of Real-Time PCR analysis exhibited that Ramalin could repress mRNA levels of IL-18, MCP-1, RANTES, and CCL17 in HaCaT cells. Furthermore, western blot analysis also displayed that the molecule resulted in the inhibition of nuclear transfer of NF-κB signaling pathways
and MAP kinase pathways. Additionally, ELISA and Real-Time PCR analysis revealed that Ramalin could suppress the level of mRNA and release IL-4. Ramalin was also able to attenuate the expression of NFAT2 in RBL-2H3 cells. Thus, overall, the study indicates that Ramalin is a potent anti-allergic agent [29].

3.7. Antiatherosclerotic

Park et al. targeted to study the effect of Ramalin against atherosclerosis in mice. Thirty-four male Apo-/- mice and seventeen control (C57BL/6) mice were kept on a fat-enriched diet including excessive cholesterol for 147 days. Ramalin (200mg/kg) was administered orally three times per week to seventeen of the Apo-/- mice which resulted in the decrement of serum levels of total cholesterol, c-Reactive protein (CRP), membrane co-factor protein (MCP-1), and oxidized low-density lipoprotein (oxLDL). Additionally, the level of serum high-density lipoprotein (HDL) was enhanced. In contrast, the molecule exhibited insignificant repressive action on the deposition of lipid and local infiltration of monocytes or macrophages in the aorta. Thus, the findings of this study indicated the protective action of Ramalin in opposition to atherosclerosis by modifying the mediators in serum capable of causing inflammation [30].

Park et al. evaluated the consequence of Ramalin on the expression of VCAM-1 (Vascular Cell Adhesion Molecule) and PADI4 (peptidylarginine deiminase IV) in HASMCs (TNF-α-induced human aortic smooth muscle cells). It was evident from the western blotting and cell adhesion assay that Ramalin could obstruct TNF-α induced adhesion of THP-1 monocytic cells and expression of VCAM-1 and PADI4 based on its dose. Additionally, the obstruction of PADI4 action by siPAFI4 repressed TNF-α induced VCAM-1 and c-Fos protein expression indicating the involvement of PADI4 in VCAM-1 expression. Additionally, the results also exhibited that Ramalin could inhibit the phosphorylation of MAPK and the nuclear transfer of NF-κB and AP-1 induced by TNF-α. The study concluded that Ramalin is a potent therapeutic candidate to decrease the danger of atherosclerosis as it could inhibit TNF-α-induced expression of VCAM-1 via the repression of MAPKs, NF-κB, PADI4, and AP-1 signaling pathway [31].

Further, a study by Park et al. demonstrated that Ramalin inhibited the VCAM-1 expression in VSMCs via the repression of MAPK and PADI4 dependent NF-κB and AP-1 signaling pathways through the induction of reactive oxygen species. It also repressed the expression of VCAM-1, which was responsible for decreasing the adhesion of THP-1 cells to TNF-α-stimulated VSMCs. Thus this study indicated the beneficial effect of Ramalin in preventing atherosclerosis [28].

3.8. Antiatopic Dermatitis

Atopic dermatitis, also known as eczema, is a skin disease where the skin becomes red and itchy. Due to such damage to the skin, there is an enhanced generation of mediators of inflammation, which are responsible for activating the immune cells, which in turn lead to the commencement of the atopic dermatitis inflammatory cycle [32]. An in-vitro and in-vivo study targeted the evaluation of the therapeutic potential of Ramalin against atopic dermatitis. When administered orally, Ramalin could decrease the scratching activity and remarkably decrease the levels of serum immunoglobulin E & IL-4 and the mRNA levels of IL-4 and IL-10 in atopic dermatitis-induced Balb/c mice. The findings of the in vitro experiments revealed that there was remarkably reduced production of inflammatory chemokines and cytokines, such as TARC, MCP-1, RANTES, and IL-8 in TNF-α-stimulated HaCaT cells. Overall, the investigation revealed that Ramalin could alter the generation of mediators of immunity by hindering the signaling pathways of MAPK and nuclear factor-kappa B. Thus, Ramalin reduced the progression of atopic dermatitis, encouraged the healing action of the Th2 cell-mediated immune reactions, and decreased the levels of mediators of inflammation in mast cells and keratinocytes [33].

3.9. Neuroprotective

A patent US 9,968,576 B2 dated May 15, 2018, was published in the United States stating the novel use of Ramalin in neurodegenerative diseases. It was stated that Ramalin could inhibit the expression of BACE1 and inflammasome. Thus, the patented invention could improve cognitive behavior by hindering the expression of an inflammatory mediator containing NLRP inflammasome protein and BACE [34].

3.10. Anti Hepatic Fibrosis

As ROS (reactive oxygen species) are involved in the pathogenesis of hepatic fibrosis, the potential of antioxidants in its treatment has been widely studied [35, 36]. Kim et al., to evaluate the beneficial effects of Ramalin in opposition to hepatic fibrosis in-vitro and in vivo, conducted a research investigation. It could inhibit the activation of the hepatic stellate cell (HSC) in-vitro, and there was a drastic decrement in the accretion of the extracellular matrix in the hepatic tissues. The compound did not produce any adverse reactions in the hepatic cells under study. When the drug was administered orally, it was seen that there was an improvement in the gross manifestation of the liver along with improved body and liver weight as compared to rats treated with dimethyl nitrosamine (DMN) injection. Moreover, all the biochemical markers of the serum also reached their normal range. The therapeutic effect of Ramalin in hepatic fibrosis (in rats induced by DMN) was probably due to repression of a-SMA (α-smooth muscle actin) and upregulation of HO-1 (heme oxygenase-1). Additionally, the investigational compound also decreased the accumulation of collagen and the levels of hydroxyproline and malondialdehyde in hepatic tissues of DMN injected rats. The positive effects of Ramalin against the succession of hepatic fibrosis were via “erythroid 2 related factors 2” (Nrf2) moderated antioxidant reaction proteins like“HO-1 and NADPH quinone dehydrogenase 1 (NQO-1)” [37].

3.11. Others

3.11.1. Autophagy Suppression

Research carried out by Park et al. showed that Ramalin could inhibit Lipopolysaccharide (LPS)-induced autophagy and inducible nitric oxide synthase (iNOS) in macrophages. The comparison of autophagy-associated gene expression in
LPS stimulated and sodium nitroprusside (SNP) stimulated macrophages was done by the method of western blotting to confirm the role of NO in autophagy. It was seen that Ramalin did not exhibit the suppression of nitric oxide and autophagy in macrophages exposed to NO donor, SNP. Additionally, the suppression of production by nitric oxide remarkably decreased the autophagy induced by LPS. The results revealed that Ramalin might repress autophagy induced by LPS by hindering the secretion of nitric oxide. Moreover, the compound could also show inhibitory action on activation of MAPK and LPS-induced NF-κB [38].

3.11.2. Enzyme Inhibition

Enzymatic inhibition is one of the therapeutic approaches utilized to manage some diseases such as obesity, cancer, diabetes, and Alzheimer’s disease. Thus, in this direction, Brewer et al. conducted a study to investigate the anti-tyrosinase action of Ramalin [16]. The compound was found to be more active than kojic acid. Ramalin was also reported to be 1.2 times more efficacious than commercially available Kojic acid in hindering the activity of the tyrosinase enzyme, which is responsible for skin cell whitening. Another study carried out by Chang et al. [39] revealed that Ramalin could inhibit melanogenesis by hindering the activity of tyrosinase by down-regulating the melanogenic proteins [40].

3.12. Toxicity Studies

Paudel et al. reported that Ramalin is not toxic in nature as it did not show any toxicity in the range chosen for the working concentrations. It exhibited almost zero cytotoxicity in human fibroblast and keratinocyte cells at its antioxidant dose [16]. Ramalin did not produce any adverse effects on hepatic stellate cells (HSC) [37]. Thus, it can be used for pharmaceuticals, including cosmetics.

Fig. (3). Molecular mechanisms of ramalin. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

CONCLUSION

The current review has summarized the multi-mechanistic approach of Ramalin in a broad range of pharmacological activities, presenting numerous scientific evidence. Ramalin has shown its potency as an antioxidant, anti-cancer, anti-inflammatory, anti-obsity, antibacterial, anti-allergic, antiatherosclerotic, antiatopic dermatitis, neuroprotective, and anti-hepatic fibrosis in autophagy suppression and enzyme inhibition are also portrayed briefly (Fig. 3). Very few studies are there regarding the toxicity studies of Ramalin, which indicate that it possesses a safety profile in the pharmacologically effective concentrations. Ramalin needs further exploration at the molecular and cellular level, multiple pathogenic processes, preclinical biological assays, and clinical trials in order to establish the potential of this molecule in the pharmaceutical field.

LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABTS•+</td>
<td>2, 2-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid</td>
</tr>
<tr>
<td>AIF</td>
<td>Adherence-inhibiting Factor</td>
</tr>
<tr>
<td>aP2</td>
<td>Adipocyte Fatty Acid-binding-Protein</td>
</tr>
<tr>
<td>aSMA</td>
<td>Alpha Smooth Muscle Actin</td>
</tr>
<tr>
<td>BACE1</td>
<td>Beta-secretase 1</td>
</tr>
<tr>
<td>Bax</td>
<td>BCL2-Associated X Protein</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>B-cell lymphoma 2</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated Hydroxyanisole</td>
</tr>
<tr>
<td>C/EBPs</td>
<td>CCAAT Enhancer-binding-Proteins</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>C57 Black 6</td>
</tr>
<tr>
<td>CCNB1</td>
<td>Cyclin B1</td>
</tr>
</tbody>
</table>
CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

The authors are thankful to the KIET School of Pharmacy, KIET Group of Institutions, and Ghaziabad for providing their constant support for research activities.

REFERENCES


The authors are thankful to the KIET School of Pharmacy, KIET Group of Institutions, and Ghaziabad for providing their constant support for research activities.


Acharias, E. Lichenographia universalis 1809, 122, 598.


