2. Literature Review

Cordyceps spp. holds paramount importance owing to its multifaceted contributions to traditional medicine, modern research, and the global health and wellness industry. With a rich history deeply rooted in traditional Chinese medicine, *Cordyceps* species, including *Cordyceps militaris* and *Cordyceps sinensis*, have been valued for their diverse therapeutic attributes. These fungi are recognized for their potential to boost the immune system, enhance energy levels, and act as adaptogens, aiding the body in management of stress. DNA barcoding method using molecular taxonomy has been widely applied for species identification of animals, plants and fungi (Seifert 2009). Certainly, the nuclear ribosomal internal transcribed spacer (ITS) region has been considered as a universal DNA barcode marker for fungal identification (Schoch et al., 2012). In recent years, *Cordyceps* has gained widespread attention for its adaptogenic and antioxidant properties, prompting extensive scientific studies into its potential applications for various health conditions. The fungi's ability to improve respiratory function, support cardiovascular health, and exhibit anti-inflammatory and anti-tumor activities has fueled its popularity in modern herbal supplements. Furthermore, *Cordyceps* has found a niche in sports and fitness communities, where it is believed to enhance endurance and stamina. The global interest in *Cordyceps* spp. reflects its dynamic role, bridging traditional information with modern scientific investigation, becoming a prominent and valuable component in the realm of natural health and wellness.

2.1 Diversity of *Cordyceps* spp.

Cordyceps spp. is disseminated in the Tibetan Plateau of China, Bhutan, and the high-altitude Himalayan grassland of India and Nepal (Shrestha et al., 2017; Giri et al., 2023). The distribution is diverse, including all regions except Antarctica, with its maximum prevalence in subtropical and tropical areas, mostly East and Southeast Asia (Sung et al., 2007). In India, the scientific information in the form of research on *Cordyceps* is very limited, though *Cordyceps* has been reported from Garhwal, along with the hilly region of the district of Pithoragargh, Uttarakhand (Caplins 2016). Sikkim is the organic state of India with rich flora and fauna. The distribution of *Cordyceps* spp. has been reported from Lachung and Lachen valley of North Sikkim and Gnathang valley in East Sikkim (Pradhan et al, 2020). In Arunachal Pradesh *Cordyceps* has been documented from alpine meadows of some protected area of Dehang-Debang Biosphere Reserve and Mechuka Valley of Tsi- yomi District (Singh et al., 2018).

2.2 Chemical composition of *Cordyceps* spp.

The *Cordyceps* spp. genus has numerous bioactive components and their derivatives, which function as secondary metabolites. The diverse range of chemical substances present in these fungus makes them highly interesting for investigating their therapeutic effects and doing pharmacological research. The main chemical constituents found in different species of *Cordyceps* include flavonoids, alkaloids, bioxanthracenes, polyketides, cyclic peptides, phenolic compounds, nucleosides, and sterols (Olatunji et al., 2018). The abundance of cyclic peptides in most *Cordyceps* spp. is particularly remarkable in comparison to other compounds. In addition, several types of *Cordyceps*, including *C. militaris*, are known for containing high levels of cordycepic acid (CA) and cordycepin. Cordycepin (2′-deoxyadenosine) and 3′-deoxyadenosine were identified in *C. sinensis* using techniques such as nuclear magnetic resonance (NMR) and infrared spectroscopy (IR) (Kaushik et al., 2020). Furthermore, *Cordyceps* spp. was shown to have a diverse range of Beta-glucans, beta-mannans, complex polysaccharides, heteropolysaccharides, cross-connected beta-mannan polymers, and saccharides, including cyclofurans composed of five and six ring carbon sugars (Das et al., 2021). Živković et al. (2019) discovered the presence of immunosuppressive chemicals, including cyclosporine, in *Cordyceps subsessilis* Petch, despite the large number of beneficial molecules detected in it. In addition, certain immunosuppressant chemicals were also extracted from the closely related *Cordyceps* species *Isariasinclairii* (Berk.) (Das et al., 2021).

2.3 Cordycepic Acid and Cordycepin

Cordycepic Acid and Cordycepin are notably originate in *C. militaris* constituting essential bioactive molecules with potential therapeutic applications Yang et al., 2020). In terms of structure, CA is defined as D-mannitol and the cordycepin as 3′-deoxyadenosine. Since cordycepin lacks the oxygen molecule at the 3rd carbon of the sugar found in ribose, it is known to be an analogue of the amino acid a derivative. Multiple extraction techniques can be utilized for this chemical, and a commonly adopted method uses a blend of water and acetonitrile in a 95:5 v/v ratio, flowing at a rate of 1.0 ml/min (Kaushik et al., 2020).

Cordycepin has been linked to a variety of therapeutic advantages, as well as effects on nucleic acids, the cell cycle, apoptosis, and intracellular targets. Its structure is alike to that of adenosine, which accounts for its diverse role in molecular activities within cells (Nxumalo et al., 2020). In contrast, CA structurally represents an isomer of quinic acid, demonstrating diverse potential medicinal applications (Das et al., 2021). Initially characterized as 1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid (Olatunji et al., 2018), CA was later identified as a crystalline substance of D-mannitol (Zhao et al., 2019). Its primary distinction from quinic acid lies in its formation of dextrorotatory instead of a lactone (Zeng et al., 2019). The content of CA in *Cordyceps* spp. exhibits considerable variation, typically ranging from 7% to 29% in *C. sinensis* and fluctuating across different growth stages of *Cordyceps* spp. (Charen & Harbord, 2020). CA exerts a significant impact on the treatment of liver fibrosis (Yan et al., 2017), functioning as a diuretic, possessing anti-free radical properties, and influencing plasma osmotic pressure (Deng et al., 2020).

2.4 Polysaccharides

Cordyceps species includes a variety of polysaccharide constituents. The pods that produce fruit of *Cordyceps* species usually have a polysaccharide content of 3-8% (Asai et al., 2012). Polysaccharides derived from *Cordyceps* species are widely acknowledged for their medicinal significance and can serve as fundamental elements in drug formulations (Tomar et al., 2022). These polysaccharides exhibit effective blood sugar level control (Abate et al., 2023), demonstrate antitumor and antimetastatic effects (Thiery et al., 2023), and possess antioxidant, immunoprotected, and anti-influenza properties. *Cordyceps* spp. polysaccharides comprise structurally various organic macromolecules with a broad range of physiochemical properties, existing in either intracellular or extracellular forms.

Polysaccharides with a molecular weight exceeding 16,000 have demonstrated effective antitumor properties (Paspati et al., 2023). *Cordyceps* spp. also have various antitumor polysaccharides that have been extracted from *C. cicadae*, *C. sinensis*, *C. militaris*, *C. ophioglossioides* (*Tolypocladiumophioglossoides*). Studies have shown that when these polysaccharides are combined with other chemotherapy medications, they have a synergistic effect and improve the body's resistance (Nurashikin-Khairuddin et al., 2022). The polysaccharides obtained from *Cordyceps* spp. mostly consist of mannan, glucan, glycoprotein, and heteroglycan. Notably, only β-(1→3) glucan, proteopolysaccharide and galactosaminoglycan derived from *C. cicadae*, *C. ophioglossioides*, and *Cordyceps* spp. have exhibited anti-tumor effects (Das et al., 2021).

2.5 Proteins and Nitrogenous Compounds

Cordyceps spp. is rich in vital amino acids, peptides, proteins, and polyamines (Das et al., 2021). In addition, *Cordyceps* spp (Das et al., 2021) contains some uncommon cyclic dipeptides, including as cyclo-[Gly-Pro], cyclo-[Leu-Pro], cyclo-[Val-Pro], cyclo-[Ala-Leu], and cyclo-[Thr-Leu]. Kumar et al. (2022) have detected substantial quantities of polyamines, including 1,3-diaminopropane, cadaverine, spermidine, spermine, and putrescine. Additional nitrogenous chemicals, such as putrescine and putrescine, were also identified (Gebremariam et al., 2022).

2.6 Nucleotides/Nucleotide Derivatives

Cordyceps spp. contains a significant number of nucleotides and their derivatives, along with other components. Nucleosides in *C. sinensis* have significant importance in therapeutic applications (Phan et al., 2018). Various nucleosides, including hypoxanthine, guanine, adenosine, uracil, uridine, guanosine, inosine, cytidine, thymidine, adenine, and cytosine, have been extracted from *C. sinensis*. Guanosine, among the nucleotide components, exhibits the highest content ratio compared to others (Zhang et al., 2022). The character of nucleosides differs between natural and cultivated *C. sinensis*.

Cordyceps spp. contains unique nucleosides not found elsewhere in nature, including hydroxyethyl adenosine, guanidine, 2′-3′-dideoxyadenosine, deoxy guanidine, cordycepin triphosphate, adenosine, and distinctive deoxy uridine structures. Cordycepin (3′-deoxyadenosine) and adenosine exhibit various functions, such as immunomodulatory and antioxidant activities. The compound cordycepin was analyzed in a sample of *C. sinensis* using NMR (nuclear magnetic resonance) and the use of infrared spectroscopy (IR). The identification of cordycepin was accomplished through the use of several analytical procedures and techniques, such as RP-HPLC (Yang et al., 2020), HPLC–ESI-MS (Chang-Liang et al., 2019), and HPLC-DAD (Nguyen et al., 2021).

2.7 Fatty Acid and Sterols

Fungi contain sterols, including ergosterol, which plays an important role in the synthesis of vitamin D2 with noteworthy therapeutic importance. *Cordyceps* spp. has been acknowledged for hosting various sterol-type compounds, including ergosterol peroxide, daucosterol, 3-sitosterol, ergosterol, campesterol, and ergosterol-3 (Das et al., 2021). The amount of ergosterol in *Cordyceps* spp. differs based on their growth stage. For example, the concentration was 1.44 mg/g in *Cordyceps* spp. mycelium and 10.68 mg/g in fruit bodies (Das et al., 2021). *Cordyceps* spp. also contain derivatives such as campesterol, 3-sitosterol, D-3-ergosterol, and daucosterol. HPLC is used in *C. sinensis* to identify ergosterol. The types of fatty acids found in *Cordyceps* spp. can be classified into two main categories: saturated and unsaturated.

The majority of the fatty acids included are unsaturated fatty acids, making up to 57.84% of the total (Yang et al., 2021). Various fatty acids, including myristic acid, lauric acid, oleic acid, stearic acid, palmitic acid, linoleic acid, pentadecanoic acid, and docosanoic acid, have been documented in *Cordyceps* spp. (Yang et al., 2009). A total of 28 fatty acids, including both saturated and unsaturated types, as well as their derivatives, were identified from *C. sinensis*. Additionally, polar compounds such as alcohols and aldehydes were also reported to have been found (Phan et al., 2018).

Unsaturated fatty acids have physiological benefits that include lowering blood lipid levels and avoiding cardiovascular illnesses. Two sterols were extracted using methanol and exhibited an anticancer activity. The structures of these sterols were characterized using 1D and 2D NMR spectroscopy (QIAN et al., 2021). The application of pressurized fluid extraction (PLE) and the conversion of trimethyl silyl (TMS), together with GC-MS analysis, uncovered the existence of cholesterol, campesterol, and β-sitosterol, similar to ergosterol, in the natural (wild) *C. sinensis* (Li et al., 2020).

2.8 Other Constituents

Apart from the fundamental constituents, *C. sinensis* primarily comprises essential amino acids, proteins, polyamines, peptides and distinctive cyclic dipeptides, such as cyclo-[Leu-Pro], cyclo-[Thr-Leu], cyclo-[Gly-Pro], and cyclo-[Val-Pro] (Das et al., 2021). Some cyclic dipeptides, specifically cyclo-(Phe-Pro) and cyclo-(Leu-Pro), have demonstrated anti-mutagenic and antimicrobial properties, particularly in combating the emergence of pathogenic yeasts and vancomycin-resistant Enterococcus (VRE) (Pellegrino et al., 2022). According to research, cyclic (bacterial) dipeptides play a role in inhibiting aflatoxin development (Nxumalo et al., 2020). There is considerable variation in protein levels among fruit bodies (30.4%), mycelial fermentation (14.8%) and dead larvae (29.1%).

The primary amino acids found in the larvae include aspartic acid, amino acid and glutamic acid (Moraga et al., 2022). Cordymine, a peptide isolated from medicinal mushrooms of *C. sinensis*, has been recognized for its anti-inflammatory and antinociceptive properties (Cao et al., 2020). The fraction of exopolysaccharide (EPSF) is obtained from the harvested *C. sinensis* supernatant. After collecting the cultured supernatant, it undergoes processing with thrice the volume of 95% ethanol for precipitation, consequential in a substantial amount of EPSFs on the soil (Zhang et al., 2019).

EPSF exhibits a broad range of pharmacologic effects, with its most significant impacts being immunomodulatory and antitumor (Gebremariam et al., 2022). Through the induction of diverse immune responses, EPSF has proven its capacity to scavenge free radicals, encourage the differentiation of cancer cells, and augment antitumor activity (Lin et al., 2022). Using size chromatography and ion-exchange, polysaccharide (PS) is isolated from *C. sinensis* mycelia that has been under cultivation. It has been noted that a polysaccharide fraction (PSF) isolated from the fungus *C. sinensis* relaxes macrophages (Nguyen et al., 2021). The transcription factor kappa-B (NF-κB) pathway is activated, this PSF can transform M2 macrophages into M1 phenotypes. PSF also has immune-modulating properties, just like other polymers (Kaushik et al., 2020). The result of *C. sinensis* on T-lymphocyte groups among individuals with chronic kidney disease were studied. It was discovered that various polysaccharide parts of *Cordyceps* spp. enhance kidney functions, cells immunity, monocyte-macrophage phagocytes function, spleen indices and thymus (Li & Kan., 2017).

2.9 Pharmacological potential of *Cordyceps* spp.

A variety of compounds entities contribute to the extensive and extraordinary pharmacological activities associated with *Cordyceps* spp. (Cunningham & Long, 2019). Notably, among the diverse range of species, *C. sinensis* stands out as the most extensively investigated, particularly in terms of researching and exploring its therapeutic potential (Cao et al., 2020). The potential use of *Cordyceps* spp. in therapy include antimicrobial, anti-inflammatory, antifungal, antitumor, immune-stimulatory, anticoagulant, anti-arteriosclerosis, ant obesity, anti-fatigue, antihyperlipidemic, anti-diabetic, anti-thrombotic, antioxidant, antibacterial, immunomodulatory, and antimalarial properties (Sun et al., 2021).

2.10 Immunoregulation of *Cordyceps* spp.

Immunoregulators are substances that aid in the regulation of the immune system. *Cordyceps* spp. has a variety of chemicals that have immunomodulatory effect. C-type lectin receptors (CLRs) and Toll-like receptors (TLRs) have a crucial function in identifying the active components of *Cordyceps* spp. during the reduced responsiveness and activation of immunomodulation in antigen-presenting cells (APCs). These components not only alter the expression of CLRs and TLRs, but they also have a substantial effect on their intracellular signaling pathways. TLRs utilise adapter proteins, such as TRIF (TIR domain-containing adapter inducing IFN-β) and MyD88 (Das et al., 2021), that cover the Toll/IL-1 receptor (TIR) domain. The bioactive components found in various species of *Cordyceps*, such as *C. cicadae* S.Z. Shing, *C. militaris*, *C. sinensis*, and *C. sobolifera* (Hill ex Watson), stimulate the Toll-like receptor 4 (TLR4) signaling pathway. This leads to the activation of the MAPK pathway and extracellular signal-related kinase 1 and 2 (ERK1/2), which in turn promotes the production of Treg/Th2 cells (Jędrejko et al., 2021).

Furthermore, the interaction between dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and TLR4 enables these biologically active components to initiate unknown intracellular pathways, resulting in the suppression of NF-κB and MyD88 activation (Kempf et al., 2010). Furthermore, these elements inhibit NF-κB activity by promoting the expression of negative regulators of TLR signaling, such as suppressor of cytokine signaling (SOCS) and phosphatidylinositol-3-kinase (PI3K), in combination with DC-SIGN-mediated rapidly accelerated fibrosarcoma (RAF) signaling (Yu et al., 2022). NF-κB has a crucial role in preparing Th1 cells.

The presence of co-receptors such as DC-SIGN and C-type lectin receptors (CLRs) increases the intricacy of the signaling pathways. The activation of the mannose receptor (MR) and macrophage galactose-type C-type lectin (MGL) has a role in the differentiation of Treg/Th2 cells, as demonstrated by Das et al. in 2021. *Cordyceps* spp. utilises the tactic of breaking down host intracellular molecules to alter the host's immune response. The polysaccharides found in *Cordyceps* spp. have a crucial function in breaking down endosomal Toll-like receptors (TLR6, TLR4, TLR3, TLR2) and host mRNA, which helps to enhance Treg/Th2 responses (Abo Nouh et al., 2021). The active bio-constituents in question commence the process of priming Treg/Th2 cells, as may be observed by the engagement of C-type lectin receptors (CLRs). The NLRP3 inflammasome, consisting of NLRP3 and caspase-1, controls inflammatory processes by releasing IL-1β and enhancing Th1 responses.

A study examined the effects of *C. sinensis* on the activity of human natural killer cells (NK) and murine activity, as well as the colony pattern of the B16 melanoma cell line in the lungs of mice. The researchers noted an augmentation of natural killer activity in mice, both in vitro and in vivo. Peripheral blood mononuclear cells (PBMCs) that were exposed to C. *sinensis* before being incubated exhibited an increase in natural killer (NK) cell activity in human PBMCs. Additionally, this treatment resulted in a reduction in the formation of B16 melanoma colonies in the lungs of mice (Dehghani et al., 2022). These data indicate that *C. sinensis* has the ability to enhance the immune response in people with weakened immune systems. In addition, the ingestion of a hot water extract made from the mycelia of *C. sinensis* resulted in the regulation of macrophages in the intestinal immune system of mice. The findings of this study demonstrated that the production of IL-6 was regulated by promoting macrophages and enhancing the secretion of hematopoietic growth factors from Peyer's patch cells, which predominantly consist of T and B cells. The growth factors examined were IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Koh et al., 2002; Song et al., 2022).

Additional research has reported *Cordyceps* spp. induced cytokine modulation. In the context of Group, A Streptococcus (GAS) infection in U937 cells, *C. sinensis* plays an immunomodulatory role by inducing the production of cytokines such as IFN-γ, IL-12, and TNF-α, ultimately enhancing phagocytosis (Shi et al., 2022). The immune activation triggered by *C. militaris* polysaccharides (CMP) was explored in cyclophosphamide-induced immunosuppressed mice, with assessments covering parameters such as phagocytic index, other biochemical factors, and lymphocyte proliferation (Tima et al., 2022), suggesting its potential as a prospective immunomodulatory agent. A compound's ability to stimulate or activate an organism's immune system explains its immuno-stimulatory activity. Various *Cordyceps* spp. species demonstrate immuno-stimulatory effects in diverse regions of the body. It has been documented that *C. sinensis* is used to treat respiratory infections by strengthening the natural defences and boosting the immune system's response (Li et al., 2021).

Cordyceps spp. also bolsters the adaptive immune system, encompassing cellular and humoral immunity (Qin et al., 2020). The sugars in poly produced by *C. gunnii* (Berk.) were tested for their immunostimulatory and anti-tumor future potential as well as their ability to induce the production of cytokines in normal, immune-compromised and H22-bearing mice. According to their studies, it is likely that polysaccharides derived from C. *gunnii* (Berk.) Berk. enhance both cellular and humoral immune responses, while also inhibiting cancer growth. CP2-S is a newly discovered polysaccharide derived from C. militaris. It has been found to possess immunostimulatory properties by enhancing phagocytosis, NO production, respiratory burst, and the release of IL-1β and IL-2 via phage secretion (Bi et al., 2020).

A prior work documented that a novel low molecular weight polysaccharide derived from matured *C. militaris* activates the immune system by triggering the MAPK pathway, NF-κB, and toll-like receptor (TLR) pathways in splenic lymphoid cells and natural killer cells, respectively. Carbon authorization in tumor-bearing mice indicated that ethanol extracts of *C. sinensis* enhanced phagocytosis activity. Furthermore, it resulted in a substantial increase in the activity of acid phosphatase and lysosomal enzymes in macrophages, suggesting that it had anti-tumor properties through an immunostimulatory mechanism (Bi et al., 2020).

2.11 Anti-inflammatory Potential of *Cordyceps* spp.

The ethanolic extract of cultured cells from *C. militaris* has potent anti-inflammatory activities and reduces the generation of inducible nitric oxide synthase (iNOS) in macrophages in carrageenin-induced edoema. The anti-inflammatory properties of this substance are reinforced by the heightened production of nitric oxide (NO) by iNOS during instances of inflammation, leading to cellular harm (Li et al., 2020). The butanolic extract of *C. militaris*, which mostly consists of cordycepin, effectively suppresses the generation of NO in macrophages stimulated by lipopolysaccharide (LPS).

Cordycepin inhibits the activation of protein kinase B (Akt), IκBα, and p38, and also suppresses TNF-α, cyclooxygenase-2 (COX-2), iNOS, and NF-κB translocation in macrophages, according to Das et al. (2010). This suggests that cordycepin has the potential to be used in the treatment of illnesses associated to inflammation (Kushairi et al., 2020). The study conducted by Ying et al. (2020) has shown evidence that *C. sinensis* enhances cell-mediated immunity. It is noteworthy that several data indicate that the use of *C. sinensis* can function as an immunosuppressive medication for post kidney transplantation, without any evident adverse effects. Furthermore, both cordycepin and *C. sinensis* regulate the actions of human immune cells in a controlled environment by controlling the expression of IL-1β, -6, -8, -10, and TNF-α in inactive cells, and by preventing the expression of IL-2, -4, -5, -12, IFN-γ, and TNF-α produced by phytohemagglutinin (Deng et al., 2022).

In addition, when cordycepin was administered to the human monocytic cell line (THP-1), it was found to increase the activity of transcription factors that play a crucial role in regulating many cytokine genes. This indicates that cordycepin has immunoregulatory properties. A study conducted by Fan et al., (2021) found that a heteropolysaccharide derived from grown *C. sinensis* shown the ability to enhance immune response in mice exposed to radiation. This was achieved by mitigating oxidative damage and modulating the production of cytokines, specifically IL-4, -5, and -17. The methanol extracted from *C. sinensis* contains immunosuppressive compounds that inhibit blastogenesis, NK cell activity, and the production of IL-2 and TNF-α by human T cells when stimulated with phytohemagglutinin. The study by Tima et al. (2022) found that Cordycepins A–E were effective in inhibiting the formation of superoxide anion and elastase release when applied to the basic extract of *C. sinensis* and its partially extracted portions.

Macrophages were subjected to treatment with varying doses of *C. militaris,* the hot water extract of fruiting bodies exhibits strong inhibitory effects on inflammatory mediators, as evidenced by reduced production of LPS-induced TNF-α, NO, and IL-6 secretion (Song et al., 2022). A study revealed similar results, showing that *C. militaris* polysaccharides (CMP) increased immunological activation. This led to improvements in spleen lymphocyte activity, thymus and spleen size, total white blood cell count, and the function of immunoglobulin G (IgG) in the serum of mice. The study conducted by Zhang et al., (2022) found that CMP increased the expression of TNF-α, IFN-γ, and IL-1β mRNA.

A separate inquiry was conducted to examine the anti-inflammatory properties of *C. bassiana* (Yang et al., 2020). The butanolic fraction of *C. bassiana* showed potent anti-inflammatory activity against LPS-activated RAW 264.7 macrophages. It effectively inhibited the activation of p38 and c-Jun N-terminal kinase (JNK), and also blocked the IκB/NF-κB pathway (Vuong et al., 2010). Moreover, anti-inflammatory substances such as 1-naphthol and 4-quinolinol were found in *C. bassiana*. The anti-inflammatory properties of the *Cordyceps* spp. strain CBG-CS-2 were discovered (Sen et al., 2023). CBG-CS-2 was reported to inhibit NF- κB along with activation of AP-1 (Activating protien) in LPS- activated macrophages which are responsible for inflammation by lowering NO, inducible nitric oxide synthase (iNOS) production and pro inflammatory cytokines (Park et al., 2015).

The activity of CBG-CS-2 has the ability to modify the response to inflammatory conditions in macrophages, indicating its possible use as a medication or supplement for inflammation. Further studies have verified the ability of CBG-CS-2, which is derived from *P. hepiali* from *C. sinensis*, to regulate the immune system and ensure safety. These studies were conducted on healthy Korean adults (Sen et al., 2023). Adenosine, cordycepin, polysaccharides, and CBG-CS-2 are the main substances that enhance immunoregulation by promoting phagocyte responses and NK-cell activity through macrophage activation. Furthermore, the anti-inflammatory properties of C. militaris have been attributed to certain cerebrosides, namely cordycerebroside A, soyacerebroside I, and glucocerebroside, as found by Lee et al. in 2021.

Cordyceps-induced immune-regulating and anti-inflammatory action is attributed to several reasons, one of which is the antibacterial activity exhibited by *C. sinensis* in animal models. This antibacterial activity is achieved by triggering the activity of macrophages. In addition, it caused the production of IL-1β, IL-10, TNF-α, serum immunoglobulins IgG1 and IgG2b, and triggered a Th1 immune response by stimulating the release of IFN-γ and IL-12 (Chen et al., 2019). Cordymin derived from *C. sinensis* has demonstrated anti-inflammatory properties, leading to a decrease in pro-inflammatory markers TNF-α and IL-1β in an inflammation model induced by carrageenan. Similarly, the compounds cordymin-1, cordymin-2, and cordymin-4, which were isolated, showed a pain-relieving effect in a model where abdominal constrictions were generated by acetic acid (Zheng et al., 2020). The extract of *C. sinensis* also affected the release of superoxide anion and elastase from human neutrophils.

The majority of the compounds demonstrated a superior anti-inflammatory activity compared against the indomethacin control. They achieved a concentration of 0.45 μg/ml for inhibiting 50% of superoxide anion formation and 1.68 μg/ml for releasing elastase, as reported by Das et al. in 2021. On the other hand, indomethacin necessitated doses of 38.32 μg/ml and 31.98 μg/ml, correspondingly. Upon closer analysis, cordycepin shown the capacity to decrease the synthesis of prostaglandin E2, NO, and pro-inflammatory cytokines in LPS-stimulated murine BV2 microglia in a dose-dependent manner. The results indicate that cordycepin has considerable promise in inhibiting inflammatory mediators in neurodegenerative disorders (Sun et al., 2020).

2.12 Antiviral effects of *Cordyceps* spp.

When mice infected with the influenza A virus were administered an acidic polysaccharide (APS) through intranasal injection, a reduction was seen in the levels of virus levels in their lungs and lung lavage fluid, leading to a rise in their survival rate. The APS was derived with an extract of *C. militaris*. APS also led to an upsurge in the levels of TNF-α and IFN-γ. RAW 264.7 murine macrophage cells enhanced the generation of nitric oxide (NO) and stimulated the expression of inducible nitric oxide synthase (iNOS) mRNA and protein. APS has been shown to upregulate the mRNA expression of cytokines, including TNF-α, IL-1β, IL-6, and IL-10. This overexpression has been found to have potential therapeutic benefits in treating influenza A virus infection by altering the immunological function of macrophages (Liu et al., 2021).

2.13 Antioxidant and Antiaging Activity

Evaluation of the anti-aging and antioxidant capabilities of cultivated *C. sinensis* water and ethanol extracts showed a moderate decrease of MDA (malondialdehyde) production but only a little effect on superoxide (Zhu et al., 2020). *C. sinensis* exhibits anti-lipid peroxidation properties and hinders the buildup of cholesteryl ester in macrophages by affecting the oxidation of LDL (Das et al., 2021). Initially, it was suggested that the adenosine concentration in *Cordyceps* spp. did not show a clear connection to antioxidative capability. However, further study has revealed that polysaccharides do have antioxidant capabilities. Based on their research (Kopalli et al., 2022), they extracted a 210 kDa polysaccharide from farmed *Cordyceps* spp. mycelia. This polymer exhibited strong antioxidative properties, leading to the conclusion that *Cordyceps* spp. offers protection against neuronal cell damage.

The polysaccharide from *C. sinensis* likely inhibits tumor development primarily by modifying the host's antioxidative action, suggestively improving SOD activity in the liver, brain, and serum, along with GPx action in the brain and liver of mice tumor (Das et al.,2021). Additionally, it markedly reduces the MDA level in the liver and brain (Long et al., 2021). Wu et al., 2011 conducted an in vitro assessment of the antioxidant action of CMhsCPS2, a polysaccharide isolated from the fruiting bodies of *C. militaris* cultivated on solid rice medium (Wu et al., 2011). The novel polysaccharide CBP-1, isolated from *C. militaris* demonstrated hydroxyl radical-scavenging abilities.

Given the association of these radicals with the pathogenesis of various diseases, the study proposed potential clinical applications of *C. militaris* as an alternative to *C. sinensis* in TCM (L. C. Li & Kan, 2017). Oxidative stress has been linked to aging by several studies (Maldonado et al., 2023). *C. sinensis* improves cognitive function, enhances the effectiveness of SOD in red blood cells, brain, and liver, boosts the activity of Na+ -K+ -ATPase in the brain, increases the potential of catalase and GPx in the blood, and reduces the activity of monoamine oxidase in the brain and the levels of MDA in the brain and liver in aged mice. These effects are achieved by enhancing the antioxidative profile and eliminating free radicals (Kushwaha et al., 2020). The treatment of D-galactose-induced senescent animals involved the use of *C. sinensis* extract. The extract of *C. sinensis* has the capacity to demonstrate antioxidant activity and improve brain function by increasing the activity of catalase, SOD, and GPx, while simultaneously decreasing the levels of lipid peroxidation and monoamine oxidase. A different species of *Cordyceps*, known as *C. guangdongensis*, has been acknowledged for its significant antioxidative stress characteristics (Chen et al., 2019).

A study conducted by Yang et al. (2021) observed that *C. guangdongensis* increased the average lifetime and duration until half of the fruit flies died in longevity tests. The polysaccharides W-CBP50, W-CBP50 I, and W-CBP50 II from cultivated *C. militaris* were subjected to structural and antioxidant studies, which demonstrated their substantial antioxidative activity (Das et al., 2021). The polysaccharide fractions (CMP-1, CMP-2, CMP-3, and CMP-4) derived from *C. militaris* had significant antioxidant properties that varied depending on their concentration (Zhu et al., 2020). In addition, a novel low molecular weight polysaccharide (CMP-1) was isolated from farmed *C. militaris*, which exhibited radical-scavenging action. Ren et al. (2020) discovered a novel polysaccharide (CMPA90–1; compound 1) from fully developed fruiting bodies of *C. militaris*, which exhibited antioxidant properties via scavenging free radicals.

2.14 Antitumor Effects

Numerous *Cordyceps* spp. genera, whether occurring naturally or cultured, have been acknowledged for their capacity to inhibit tumor growth attributed to a variety of bioactive compounds such as polysaccharides, sterols, and adenosine (Chen et al., 2019). A glycosylated ergosterol identified in the methanolic extract of *C. sinensis* was reported as a potent antiproliferative compound effective against various tumor cell lines (QIAN et al., 2021). Furthermore, the aqueous extract of *C. sinensis* has been demonstrated to enhance Kupffer cell-mediated phagocytosis, thereby preventing metastasis (Wei et al., 2017).

A comparative study found that artificially cultured *Cordyceps* spp. demonstrate more potent anticancer activity against MCF-7, B16, HL-60, and HepG2 cancer cell lines in comparison to naturally occurring *Cordyceps* spp. (Chen et al., 2020). Cordycepin inhibits the growth of cancer cells by stimulating adenosine A3 receptors, which then affects the signalling cascade including the activation of glycogen synthase kinase three beta (GSK3β) and the suppression of cyclin D1 (Zheng et al., 2020). A study conducted on MA-10 murine Leydig tumour cells found that cordycepin-induced apoptosis was mediated through the caspase-9, 3, and 7 dependent pathways (Kopalli et al., 2022). The antiproliferative effect of cordycepin is known to occur through the involvement of two signalling pathways, namely the mammalian target of rapamycin (mTOR) and 5′AMP-activated protein kinase (AMPK) signalling (Liu et al., 2021). Cordycepin induces apoptosis in human colorectal cancer cells by enhancing the activity of B-cell lymphoma 2 (Bcl-2, proapoptotic molecules), JNK, and p38 kinase (R. F. Li et al., 2021).

When used as an adjuvant, a low concentration of cordycepin increases the chemosensitivity of gall bladder cancer cells to gemcitabine and 5-fluorouracil, potentially through the regulation of AMPK/mTORC1 signaling and the downregulation of multiple drug-resistant/hypoxia-inducible factor 1 (MDR/HIF-1α). (Lee et al., 2021). Therefore, it is confirmed that there are several routes specific to different cell types in the cordycepin-induced antitumor profile. A study conducted by Long et al. (2021) investigated the combined impact of fermented *C. sinensis* and selenium on uterine cervix cancer. The study revealed that this combination reduces oxidative stress and enhances immune function more effectively than their solo effects. The water-based extract derived from *C. militaris* a different species, had cytotoxic effects against stomach cancer (SNU1), colorectal adenocarcinoma (SUN-C4), and hepatocellular carcinoma (SNH-354). Cordycepin was identified as the active compound responsible for these effects (Kopalli et al., 2022).

The extract from *C. militaris* demonstrates antiangiogenic capabilities, as demonstrated by its ability to suppress tube formation in endothelial cells and reduce the activity of matrix metallopeptidase (MMP), a factor associated with metastasis and invasion (Yoo et al., 2004). *Cordyceps militaris* triggers apoptosis in human breast cancer cell lines by causing mitochondrial malfunction and activating caspases (Shi et al., 2022). In addition, the extracts of *C. militaris* have been found to contain pure chemicals that have been shown to inhibit the growth of PC-3, colon 205, and HepG2 cells (Rao et al., 2010). Additionally, a study by Sun et al. (2020) found that *C. militaris* effectively suppresses the growth of cancer cells in a mouse model with murine T cell lymphoma (RMA) cell-derived malignancies. This inhibition is achieved through the modulation of p85/Akt-dependent or GSK3β-related caspase-3-dependent apoptosis.

Studies have recorded that *C. sinensis* effectively suppresses the growth of tumour cells in many cancer cell lines, such as Jurkat, HepG2, PC 3, Colon 205, and MCF-7 (Deng et al., 2022). The extract of *C. militaris* and cordycepin induce programmed cell death through the activation of caspase-7, -8, and -9. This process is accompanied by an elevation in the ratio of Bcl-2-associated x protein (Bax) to Bcl-2 protein expression and a reduction in X-linked inhibitor of apoptosis protein (XIAP). These findings provide evidence for the anti-cancer effects of the extract (Nandi et al., 2024). Cordycepin demonstrated anti-cancer properties against B16 murine melanoma via stimulating the adenosine A3 receptor and subsequently activating glycogen synthase kinase-3β. Additionally, it suppressed cyclin D1 expression (Das et al., 2021). In addition, cordycepin demonstrates a synergistic impact with other medications, such as 2′-deoxycoformycin, resulting in a three hundredfold increase in the anti-cancer activity in B16 cells (Li et al., 2020). Additional mechanisms by which *Cordyceps* spp. exhibit anti-cancer effects include autophagy and apoptosis, as demonstrated in LNCaP cells, which are a type of human prostate carcinoma. In addition, the autophagy process was seen by the augmentation and buildup of microtubule-associated protein light chain-3 (LC3) (Li et al., 2020).

2.15 Hypoglycemic Activity

A study observed that polysaccharides derived from the grown mycelium of *C. sinensis* (CS-F30) effectively lower the level of plasma glucose in both normal and streptozotocin (STZ)-induced diabetic mice when administered intraperitoneally. However, when administered orally, the reduction in plasma glucose level is only slight (Stojkovic et al., 2019). In addition, Das et al. (2021) confirmed that CS-F30 enhances the functioning of hexokinase, glucokinase, and glucose-6-phosphate dehydrogenase, consequently enhancing glucose metabolism, which is accountable for its hypoglycemic effects.

The study conducted by Shang et al. (2020) examined the effects of intraperitoneal administration of CS-F10, a purified polysaccharide derived from the hot water extract of cultured mycelium of *C. sinensis*, on normal, STZ-induced diabetic, and epinephrine-induced hyperglycemic mice. The results showed a reduction in plasma glucose levels and an increase in hepatic glucokinase activity. CordyMax™ Cs-4, a product of industrial fermentation, is developed from a unique mycelial strain found in natural *C. sinensis*. It has been reported to effectively reduce baseline blood glucose and plasma insulin levels (Das et al., 2021). Furthermore, it enhances glucose metabolism by augmenting insulin sensitivity and improving oral glucose tolerance.

A study concluded that *Cordyceps* spp. exhibits hypoglycemic effect in diabetic rats caused by nicotinamide (NA) and STZ. This is evidenced by the reduction of polydipsia, hyperglycemia, and weight loss observed in the rats (Sun et al., 2019). Studies have shown that *C. sinensis* can improve the resilience of β-cells in mice with diabetes mellitus-II. The study conducted by Hao et al. (2020) has already demonstrated that *C. sinensis* provides a protective effect on podocytes in rats with diabetic nephropathy. Furthermore, a recent study by Chen et al. (2019) has demonstrated that CmNo1, a combination of the fruiting body and mycelia of *C. militaris*, has been proven to provide protection to the kidneys in mice with type 2 diabetes produced by a high-fat diet and STZ-NA. An investigation demonstrated that the aqueous extract of *C. militaris* (CMW) stimulates the expression of hepatocyte nuclear factor (HNF)-1α, which in turn activates GLUT2 to facilitate the uptake of glucose in liver cells (Ying et al., 2020). A recent work was conducted to extract and characterise cerebrosides from *C. militaris* (L.) that exhibit anti-PTP1B action. The findings demonstrated that all four cerebrosides derived from *C. militaris* displayed inhibitory effects on PTP1B, as reported by Das et al. (2021).

2.16. Vasorelaxation Hypocholesterolemic and Hypotensive Activities

Previous investigation has emphasized the influence of *C. sinensis* on cardiovascular well-being. It was discovered that a protein constituent in *C. sinensis* had the ability to reduce the average artery pressure in rats. The observed impact is ascribed to the stimulation of nitric oxide (NO) and endothelium-derived hyperpolarizing factor, resulting in a direct relaxation of the blood vessels through the endothelium. The observed hypotensive and vasorelaxation effects were attributed to either a solitary active component or the synergistic action of numerous compounds present in the extract (Hu et al., 2019).

C. sinensis not only has antioxidant qualities but also exhibits effective anti-lipid peroxidation effects. By inhibiting LDL oxidation, it successfully stops macrophages from producing more cholesteryl ester. Subsequent research examined the aqueous extract derived from cultivated CMW and its influence on serum lipid and lipid peroxide levels in a mouse model of atherosclerosis. Their research determined that CMW inhibits the accumulation of cholesterol in the aorta by hindering the oxidation of LDL through the removal of free radicals (Lin et al., 2021). In addition, a distinct investigation examined the impact of cordycepin derived from *C. militaris* on vascular diseases and rat aortic smooth muscle cells (RASMCs), specifically focusing on neointimal development. According to the study conducted by Mustafa et al. (2022), it was shown that cordycepin hinders the movement and multiplication of RASMCs (smooth muscle cells) triggered by platelet-derived growth factor-BB (PDGF-BB). This effect is achieved by interfering with the adenosine receptor-mediated NOS (nitric oxide synthase) pathways.

The interference led to a decrease in the production of neointima, indicating that cordycepin may have a potential role as an agent to combat atherosclerosis. Furthermore, it was found that cordycepin contributes to the regulation of lipid profiles without inducing toxicity. The study conducted by Kalita et al. (2022) shown a rise in the activity of lipoprotein lipase (LPL) and hepatic lipase (HL) due to the administration of cordycepin. Collectively, these studies shed light on the diverse cardiovascular benefits of *C. sinensis* and cordycepin, ranging from blood pressure regulation to the prevention of atherosclerosis and lipid profile management. In the modern treatment approach for managing both depression and diabetes, showed promising results in vanadium-enriched *C. sinensis* (VECS). A study conducted that the delivery of VECS significantly reduced sugar levels while along with increase in serum insulin.

The study showed a significant decrease in inactivity and a corresponding increase in climbing and swimming activity in diabetic rats after VECS administration. This result reinforces a modern therapeutic approach that promotes a proactive attitude towards managing both depression and diabetes (Prabhakar, 2020). A study has enhanced the comprehension of the medicinal capacity of *Cordyceps*. The polysaccharide derived from *C. militaris* that demonstrated significant antioxidant, antihyperlipidemic, and Hepatoprotective effects. This was demonstrated by a decrease in liver and blood lipid levels, as well as an enhancement in antioxidant activity and glutamate pyruvate transaminase. Studies are revealing the elements responsible for the hypocholesterolemic, hypotensive, and vasorelaxation effects induced by *Cordyceps* (Panda & Luyten, 2022). These findings contribute to the existing knowledge that supports the therapeutic capacity of *Cordyceps* in the treatment of diabetes, depression, and related disorders.

2.17 Larvicidal Activity

Cordyceps spp. is an invaluable asset because it is environmentally safe and has few or no negative effects when used as pesticides, making it highly advantageous (Qasim et al., 2020). Notably, research highlighted the insecticidal properties of cordycepin derived from *C. militaris*. This compound demonstrated effectiveness against *Plutellaxylostella* L. larvae through a direct effect, distinct from inhibiting chitin synthesis, and exhibited stomach action. Moving beyond its role as a pesticide, *Cordyceps* spp. has a rich history of medicinal use (Rocha et al., 2022). For centuries, it has been employed to enhance physical stamina, particularly in populations dealing with weakness and fatigue at high altitudes. The mushroom gained prominence in 1993 when world-class athletes revealed its inclusion in their strategies for success, incorporating a *Cordyceps* spp. based diet.

The cause of this effect is ascribed to its capacity to enhance cellular ATP, consequently augmenting bioenergy and promoting effective oxygen utilisation (Deng et al., 2020). In addition, athletes have adopted the use of *Cordyceps* spp. to counteract fatigue and debility, thereby enhancing energy levels and prolonging stamina. A study was conducted on CordyMax™ Cs-4, which is a result of mycelial fermentation of *C. sinensis*. The study revealed that CordyMax™ Cs-4 has a considerable effect on energy metabolism (Das et al., 2021). The study found that CordyMax significantly enhanced the bioenergy status in the liver of mice by increasing the level of β-ATP (adenosine triphosphate), so confirming its ability to promote energy production (Das et al., 2021). Moreover, the antioxidant properties of *Cordyceps* spp. aid in improving energy metabolism in mitochondria, hence promoting effective utilisation of oxygen and increasing the anaerobic threshold (Subramaniyan et al., 2023). The findings highlight the various advantages of *Cordyceps* spp., including its ability to act as an insecticide, its historical usage in medicine, and its potential to enhance energy levels and alleviate weariness.

The association between fatigue and depression is widely recognized, which led to a study that employed the tail suspension test in mice to evaluate the antidepressant properties of the supercritical fluid extract (SCCS) of *C. sinensis*. The results indicate that SCCS may produce a mood-enhancing effect via impacting the adrenergic and dopaminergic systems, rather than exerting an influence on the serotonergic system (Das et al., 2021).

A study was conducted to investigate the effects of Cs-4 on the aerobic capacity of healthy senior volunteers. The study, which was double-blind and placebo-controlled, found that administering Cs-4 for a period of 12 weeks increased both the ventilatory and metabolic thresholds. The study conducted by Cao et al. (2020) found that higher thresholds in elderly human volunteers are indicative of enhanced aerobic capacity without experiencing tiredness. The study conducted by Das et al. (2021) investigated the impact of polysaccharides derived from *C. sinensis* mycelium on physical tiredness in mice. The results showed that these polysaccharides increased the duration of intensive swimming, raised glycogen levels in the liver and muscles, and decreased levels of blood lactic acid and blood urea nitrogen (BUN). The observations confirmed the fatigue-reducing benefits of *C. sinensis* polysaccharides. The exercise endurance-promoting activities of *C. sinensis* are attributed to the upregulation of skeletal metabolic regulators, such as AMPK, peroxisome proliferator-activated receptor gamma (PGC)-1, and peroxisome proliferator-activated receptors (PPAR), as reported by He et al. (2020). In addition, the researchers observed the activation of the NRF-2-ARE pathway, which helped decrease oxidative stress and inflammation caused by exercise (Das et al., 2021).

When considering another species, *C. angiogenesis*, its ability to replenish, as demonstrated by the increased swimming time in mice. This comprehensive review highlights the wide range of anti-fatigue and depressive characteristics displayed by different species of *Cordyceps*, providing insight into their potential therapeutic uses. Moreover, the fatigue-reducing impact caused by *C. angiogenesis* is ascribed to a polysaccharide that lessens the buildup of blood lactic acid levels, thereby mitigating fatigue (Zhang et al., 2021). Both wild and farmed mycelia of *C. sinensis* have shown the capacity to improve motor coordination, boost respiratory and metabolic activities, and consequently enhance muscle endurance or anti-fatigue activity.

Simultaneously, these activities contribute to mood elevation and antidepressant-like effects, mitigating endogenous depression (Holliday, 2017). The antioxidative properties of *C. sinensis* are believed to underlie the amplified skeletal muscle action observed. Moreover, *C. militaris* plays a role in fatigue recovery mainly through the establishment of AKT/mTOR and AMPK pathways. Furthermore, it controls the quantities of hormone in the bloodstream. This comprehensive study examines the various mechanisms that contribute to the anti-fatigue and antidepressant effects of *Cordyceps*. It demonstrates the potential of diverse species of *Cordyceps* in resolving fatigue-related issues and improving mental health (Olatunji et al., 2018).

2.18 Aphrodisiac Potential

Cordyceps spp. is a significant source of potent energy, making it an attractive option for use in sexual stimulation and treating sexual dysfunction (Shashidhar et al., 2013). Referred to as the "Himalayan Viagra" by experts (Kashyap et al., 2016), this substance is widely acknowledged for its ability to regulate sexual hormones such as testosterone, oestrogen, and progesterone. As a result, it is highly beneficial in managing reproductive activity and repairing compromised functions (Prasain, 2013). *Cordyceps* spp. promotes the synthesis of testosterone and raises plasma testosterone levels by activating the PKA and PKC signal transduction pathways, even in sexually inactive mouse models (Lee et al., 2021). It is important to highlight a study which found that *C. sinensis* increased the growth of prostate cancer cells in mice by increasing the production of testosterone and the expression of androgen receptors (Olatunji et al., 2018). The study investigated the effects of *C. sinensis* and its extracted fractions on testosterone production in mice, using both in vivo and in vitro methods (Kaushik et al., 2020).

Furthermore, there is evidence indicating that *C. sinensis* and its extracts have a significant impact on the production of testosterone in mice, resulting in a notable increase. The provided summary emphasises the complex involvement of *Cordyceps* spp. in regulating hormones, with a particular focus on its potential uses in promoting sexual health and reproductive well-being (Nguyen et al., 2021). Administering cordycepin has significant impacts on male reproductive health, such as an augmentation in epididymis weight, improvement in sperm motility and mobility, and an increase in the quantity of mature sperm (Sharma et al., 2017). This enhances both the quality and quantity of sperm as a whole. The study offers valuable insights into the molecular aspect, indicating that protein kinase C (PKC) may have a crucial function in the steroidogenesis caused by *C. sinensis* in primary rat adrenal cell cultures. In addition, *C. sinensis* stimulates the production of steroids in primary mouse Leydig cells, leading to death in MA-10 mice Leydig tumour cells in a dose-dependent manner (Deng et al., 2022).

Specifically in MA-10 mouse Leydig tumour cells, *C. sinensis* promotes the production of steroidogenic acute regulatory (StAR) protein, a crucial element in steroidogenesis (Das et al., 2021). In their research (Bi et al., 2020), the authors investigated the impact of *C. sinensis* on steroid production in normal Leydig cells. They observed different effects on steroid production triggered by human chorionic gonadotropin (hCG) between normal and tumour cells. The results demonstrated a notable increase in testosterone production, highlighting the importance of creating new proteins for the process of steroidogenesis. The species *C. sinensis* and its separated components have the ability to increase the synthesis of testosterone, both in living organisms and in laboratory settings. Further investigating the process, we examined the effect of inhibitors targeting the PKC or PKA pathways on Leydig cells in relation to steroidogenesis triggered by *C. sinensis*. This comprehensive study offers valuable insights into the complex influence of *C. sinensis* on male reproductive parameters, illuminating prospective uses for improving male fertility. *C. sinensis* has been found to have complex effects on reproductive processes, as evidenced by multiple research (QIAN et al., 2021; Qin et al., 2020; Sun et al., 2020; Wei et al., 2017). The study conducted by Zhang et al. (2022) revealed that *C. sinensis* enhanced the cAMP-protein kinase A (PKA) signalling pathway in pure mouse Leydig cells. This activation resulted in the inhibition of P450 side-chain cleavage enzyme (P450scc) activity and a decrease in steroidogenesis triggered by human chorionic gonadotropin.

However, in MA-10 murine Leydig cancer cells, alternate mechanisms indicate that *C. sinensis*-induced steroidogenesis may potentially include the concurrent activation of both the PKA and protein kinase C (PKC) pathways (Phan et al., 2018). Additional investigation uncovered that the process of steroidogenesis triggered by *C. sinensis* requires de novo protein synthesis, elevated expression of steroidogenic acute regulatory protein mRNA, a calcium signal, and a mitochondria electrochemical gradient (Das et al., 2021). *C. sinensis* was found to enhance the production of E2 in human granulosa-lutein cells (GLCs) by increasing the expression of important enzymes, specifically StAR and aromatase. This effect also extends to the female reproductive system (Zhao et al., 2019).

C. sinensis emerges as a highly promising contender for augmenting female fertility. In addition, there have been reports indicating that *C. sinensis* and its fractions have the ability to increase plasma corticosterone levels in both mature and immature mice. Furthermore, the beneficial impacts of *C. sinensis* on the functions of reproduction and the structure of the testes in mice. These findings highlight the various and intricate effects of *C. sinensis* on reproductive processes in both males and females. The study examined the impact of extracted fractions from *C. sinensis* mycelium on testosterone synthesis in purified normal mouse Leydig cells treated with hCG in a laboratory setting. The effects of these fractions were evaluated using previous studies conducted by Charen & Harbord (2020), Holliday (2017), Wei et al., 2017, and Yan et al., 2017. The results indicated that, in contrast to the stimulating effects reported in tumour cells when treated with hCG, all extracts of *C. sinensis* reduced hCG-induced testosterone synthesis in normal mouse Leydig cells (Das et al., 2021). The difference in response is due to the activation of various receptor subtypes in normal and tumour cells, resulting in different cellular activities (Baral, 2017; Chaubey et al., 2019; Shrestha et al., 2017).

In addition, the use of *C. militaris* mycelium has been shown to have beneficial effects on both the quality and quantity of sperm. This is supported by improvements in the proportion of sperm cells that are able to move and the overall shape of the sperm, as observed in studies conducted by Nxumalo et al., 2020 and Pal & Misra (2018). Furthermore, the researchers investigated the impact of Cordycepin on primary mouse Leydig cell steroidogenesis, both in vitro and in vivo. The findings revealed that Cordycepin not only elevated the levels of testosterone in the blood, but also accelerated the production of testosterone in mouse Leydig cells cultured in a laboratory setting (Das et al., 2021). The process entails cordycepin binding to adenosine receptors, which triggers the cAMP-PKA-StAR pathway, leading to the stimulation of steroidogenesis in mouse Leydig cells (Thakur, 2020; Zhang et al., 2020). This comprehensive analysis examines the various effects of *Cordyceps* species on testosterone synthesis and sperm quality, providing insights into their prospective uses in the field of reproductive health.

Moreover, research has indicated that cordycepin has two distinct effects: it enhances the generation of progesterone and activates androgen receptors (AR) in MA-10 mice Leydig tumor cells. This dual action leads to the simultaneous initiation of steroidogenesis and death (Olatunji et al., 2018). Further investigations focused on the intracellular signaling pathways that are responsible for the effects induced by cordycepin in these cells. The phospholipase C/protein kinase C (PLC/PKC) and MAPK signal transduction pathways were found as crucial factors in both steroidogenesis and cell death triggered by cordycepin, as reported by Cao et al. (2020).

In contrast, the prolonged use of cordycepin was discovered to reverse the deterioration of testicular function in middle-aged rats (Deng et al., 2020). This indicates that cordycepin may have a possible therapeutic purpose in reducing age-related alterations in testicular function. In addition, C. militaris has shown substantial protective benefits on testicles that have been exposed to oxidative damage induced by bisphenol A, a commonly used plasticizer. *Cordyceps militaris* inhibited the decline of serum testosterone and luteinizing hormone levels caused by bisphenol A. The observed protective benefits were ascribed to the activation of crucial enzymes, such as Star, CYP11A1, 3β-HSD, and CYP17A1 expressions (Chen et al., 2019). This comprehensive analysis highlights the complex and diverse effects of *Cordyceps* species on testicular function and reproductive health.

2.19 Renal Protection

The kidney, being the principal organ responsible for the filtration and elimination of waste through the production of urine, has potential therapeutic uses in the components of *C. sinensis*. Notably, *C. sinensis* demonstrates its value in regulating kidney imbalances, such as reducing hematuria and proteinuria, with evident tissue restoration observed through histological analysis (Deng et al., 2020). Additionally, it proves beneficial in supporting kidney transplantation when combined with drugs like cyclosporin A.

This combination is especially beneficial because elevated doses of cyclosporin A have the potential to cause renal impairment (Charen & Harbord, 2020). Moreover, *C. sinensis* demonstrates nephroprotective characteristics, reducing harm caused by aminoglycosides and broad-spectrum antibiotics (Zhu et al., 2022). The observed protective effects are linked to elevated levels of 17-hydroxy-corticosteroid, 17-ketosteroid, superoxide dismutase (SOD) enzymes, and the process of free radical scavenging (Yan et al., 2023). It is important to recognize that the cultivation of *Cordyceps* spp. is a very new area of study, and there is minimal information available regarding the best conditions for growth and the impact of cultivation on the bioactive chemicals found in *Cordyceps* spp. extracts.

Additionally, concerns exist about the sustainability of wild harvesting and the potential environmental impact of commercial cultivation. Advanced research is imperative to comprehensively understand the healing effects of *Cordyceps* spp. and to create safe and effective interventions using this fungus. Future research directions should prioritize identifying optimal cultivation conditions and understanding cultivation effects on bioactive compounds in *Cordyceps* spp. extracts. This will facilitate the development of consistent, high-quality *Cordyceps* spp. extracts with standardized levels of bioactive compounds. Furthermore, it is imperative to prioritize research efforts towards establishing the safety and effectiveness of *Cordyceps* spp. in human subjects, particularly in relation to potential drug interactions.

Clinical trials are necessary to establish appropriate dosages and treatment regimens for different medical conditions. In this way, the full potential of *Cordyceps* spp. can be harnessed for therapeutic applications with a solid foundation in scientific understanding and clinical evidence. The cultivation of *Cordyceps* spp. is still in its early stages, considered by a lack of evidence regarding the optimal conditions for growth and the effects of cultivation on the bioactive components present in *Cordyceps* spp. extracts. This scarcity of knowledge is compounded by concerns about the sustainability of wild harvesting and the potential environmental impact associated with commercial cultivation. Consequently, there is a pressing need for advanced research to address these gaps. A thorough exploration of the therapeutic potential of *Cordyceps* spp. and the development of safe and effective interventions rely on a deeper understanding gained through further investigation.

To address these knowledge gaps, future research avenues must prioritize the identification of optimal cultivation conditions for *Cordyceps* spp. and an in-depth examination of how cultivation affects the bioactive components present in *Cordyceps* spp. extracts. Such endeavors are pivotal for establishing consistent, high-quality extracts with standardized levels of bioactive compounds. Moreover, it is imperative to delve into the safety and efficacy of *Cordyceps* spp. in human applications, including potential interactions with concurrent medications. The indispensable next step involves conducting clinical trials to determine appropriate dosages and treatment regimens tailored to different medical conditions. This strategic approach is paramount for unlocking the full therapeutic potential of *Cordyceps* spp. and ensuring its responsible integration into medical practices.