

RHINACANTHIN-C: A TARGETED REVIEW ON ITS PHARMACOLOGICAL PROPERTIES AND PROSPECTS FOR DRUG DEVELOPMENT

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Abstract

Rhinacanthin-C, a naphthoquinone ester derived from *Rhinacanthus nasutus* (L.) Kurz, has garnered attention due to its broad spectrum of pharmacological properties, particularly in areas such as anticancer, anti-inflammatory, antidiabetic, and neuroprotective activities. As the major bioactive compound of *R. nasutus*, rhinacanthin-C shows potential as a therapeutic agent for chronic diseases including diabetes, cancer, and neurodegenerative disorders. Recent studies have further explored its pharmacokinetics, safety, and toxicity profiles, highlighting its efficacy and possible clinical applications. Despite its potency, one challenge in the development of rhinacanthin-C for therapeutic use is the cost and complexity of its extraction and purification processes. A promising alternative is the use of rhinacanthins-rich extract (RRE), which offer comparable pharmacological benefits with a low-cost of production. This review illustrates the current findings on rhinacanthin-C, discussing its therapeutic potential, methods for standardization, and prospects for pharmaceutical applications.

Keywords: Extraction; Lead compound; Naphthoquinone ester; Rhinacanthin-C; Standardization

Introduction

Rhinacanthus nasutus is a shrub native to Southeast Asia, India, and China. It is a medicinal plant whose aerial parts and roots have been effectively used to treat herpes and other skin infections (Bukke *et al.*, 2011). The extracts of *R. nasutus* have been used for various pharmacological applications, including anti-fungal, antibacterial, antiviral, anticancer, and

antiplatelet aggregation (Sendl *et al.*, 1996; Panichayupakaranant *et al.*, 2009; Puttarak *et al.*, 2010; Bukke *et al.*, 2011; Boueroy *et al.*, 2018). In addition, they have been used in various plant-based health products, such as shampoos, soaps, lotions, sprays, and infusion teas (Suksawat and Panichayupakaranant, 2023).

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The major bioactive compounds found in *R. nasutus* are 1,4-naphthoquinone esters, namely rhinacanthins. Initially, rhinacanthin-A and rhinacanthin-B have been identified from the roots of *R. nasutus*, of which rhinacanthin-B exhibited significant cytotoxicity against human epithelial carcinoma (KB) cells (Wu *et al.*, 1988). Subsequently, rhinacanthin-C has been identified as an antiviral compound from the aerial parts of *R. nasutus* (Sendl *et al.*, 1996). Afterwards, various naphthoquinone esters, i.e., rhinacanthin-D, -G, -H, -I, -J, -K, -L, -M, -N, -O, -P, -Q, -R, -S, -T, -U, and -V, have been identified in *R. nasutus* (Wu *et al.*, 1988; Sendl *et al.*, 1996; Boonyaketguson *et al.*, 2018). Amongst these, rhinacanthin-C has been reported as the major and the most potent bioactive compound of *R. nasutus* (Panichayupakaranant *et al.*, 2009; Suksawat and Panichayupakaranant, 2023). Therefore, rhinacanthin-C plays an important role in the therapeutic effect of *R. nasutus* and has been used as a bioactive marker for the standardization of *R. nasutus* extracts (Jayapriya and Gricilda, 2015).

Although antimicrobial effects of rhinacanthin-C have been described based on the traditional uses of *R. nasutus* (Panichayupakaranant *et al.*, 2009; Puttarak *et al.*, 2010), the other therapeutic potentials of rhinacanthin-C should be emphasized to explore its broader pharmacological effects and mechanisms of action (Kumar *et al.*, 2021). This review aims to summarize and illustrate the current findings on rhinacanthin-C with regard to its potential uses as a promising therapeutic agent, encompassing the physicochemical properties, pharmacological effects, pharmacokinetic data, mechanisms of action and signaling pathways, as well as safety and toxicity assessment. In addition, relevant evidence in support of the applications of rhinacanthin-C extracts in health aspects, including green extractions and quantitative analysis of rhinacanthin-C extracts, was also provided.

Based on this review, the keywords *Rhinacanthus nasutus*, rhinacanthin, rhinacanthin-C, naphthoquinone, antimicrobial, antibacterial, antifungal, antiviral, anticancer, antitumor, anti-inflammation, anti-diabetic, neuroprotective, biosynthesis, pharmacokinetic, toxicity, extraction, and standardization were searched in the online search engines, including Web of Science, Scopus, PubMed, ScienceDirect, and Google, to obtain 30 recently published research articles, which have been published between 2014 and 2023.

Rhinacanthin-C

Rhinacanthin-C, or its IUPAC designation as [3-(1-hydroxy-3,4-dioxonaphthalen-2-yl)-2,2-dimethylpropyl]

(2E,6E)-2,6-dimethylocta-2,6-dienoate (Figure 1), is a member of the 1,4-naphthoquinone ester group with the chemical formula of $C_{25}H_{30}O_5$ and a molecular weight of 410.5. Rhinacanthin-C was first identified using the Electron ionization mass spectroscopy (EIMS) and Nuclear magnetic resonance (NMR) techniques (Sendl *et al.*, 1996). Rhinacanthin-C has a reddish-yellow semisolid oil appearance and can be dissolved in chloroform, ethanol, ethyl acetate, methanol, and hexane. It contains one hydrogen bond donor and five hydrogen bond acceptors (PubChem, 2023).

Rhinacanthin-C is naturally found in *R. nasutus*. It is markedly accumulated in the roots (3.4%-4.1% w/w) and leaves (2.5%-3.2% w/w) of this plant (Suksawat and Panichayupakaranant, 2023). At present, the full description of the biosynthetic pathway of rhinacanthin-C remains incomplete. Nonetheless, there was a review of rhinacanthin biogenesis (Panichayupakaranant *et al.*, 2021). Briefly, the chemical structure of rhinacanthin-C consists of two core skeletons, namely the naphthoquinone and isoprenyl units. Its naphthoquinone component is synthesized via the *o*-succinylbenzoate (OSB) pathway, starting with chorismic acid transformation. This pathway involves multiple steps, including enzyme-mediated reactions, ring formation via Claisen-like condensation, and several chemical modifications, such as oxidation and hydroxylation, ultimately leading to the formation of isoprenylated hydroxynaphthoquinone. The isoprenyl unit of rhinacanthin-C is produced via the mevalonate pathway, where dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) are converted into geranyl pyrophosphate (GPP) by geranyl pyrophosphate synthase. GPP and its isomers then undergo prenylation. This intermediate is subsequently transformed into aldehyde hydroxynaphthoquinone, which is reduced to form the naphthoquinone unit of rhinacanthin-C and then forming rhinacanthin-C through esterification. Summarily, the biogenesis of the naphthoquinone unit in rhinacanthin-C predominantly involves the OSB pathway, leading to the formation of isoprenylated hydroxynaphthoquinone, which integrates into its core structure (Figure 1).

Extraction and Standardization

Medicinal plants are the most abundant resource of medications used in ancient systems of medicine, contemporary medicines, nutraceuticals, dietary supplements, and chemical pharmacophores for synthesized drugs. The World Health Organization

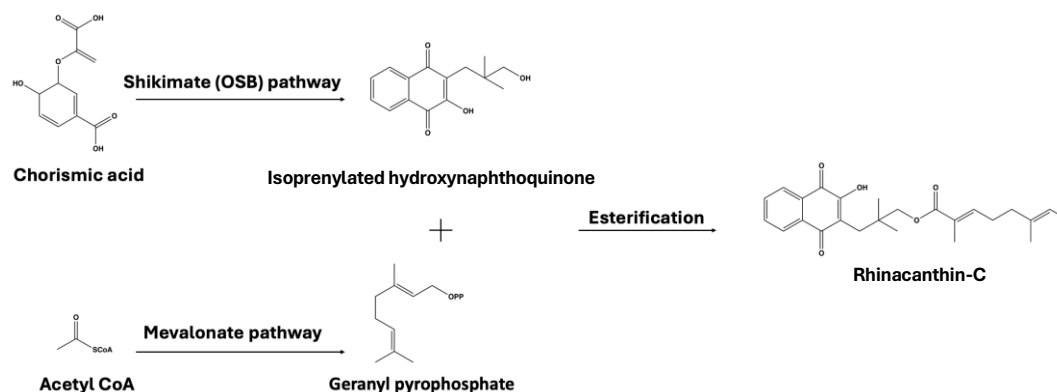


Figure 1. The biogenetic pathway of rhinacanthin-C

(WHO) encourages, advises, and promotes traditional treatments in national health care systems because they are readily accessible, low-cost, and safe (Kumari and Kotecha, 2016). The extraction is the first step in obtaining a desired bioactive chemical from plant materials. However, there is a distinction between providing a pure phytochemical and the same chemical in the form of a plant extract. It applies in the context of chemical complexity if the pharmacological impact of a chemical combination is greater than the arithmetic sum of the effects of individual components. This is the foundation for making an active constituent-enriched herbal extract. The extraction process is crucial because plants accumulate bioactive constituents in tiny amounts, embed them within the plant matrix, and often complex them with other compounds. Rhinacanthin-C, for example, is not commercially available. Moreover, the purification of rhinacanthin-C is a multistage process that takes a substantial number of hazardous solvents as well as time and energy, resulting in increased manufacturing costs. Another strategic approach that may have a synergistic impact is to use rhinacanthins-rich extract (RRE), a semi-purified extract of *R. nasutus*.

Although ethyl acetate tends to be the optimum solvent for *R. nasutus* extraction, it is an organic solvent that may be detrimental to humans and the environment. As a result, a simple green extraction that is environmentally friendly and suited for pharmaceutical applications in terms of safety and cheap cost has been established for the preparation of RRE (Panichayupakaranant *et al.*, 2009). A microwave-assisted extraction that consumed less energy and time was utilized. The process solely utilized green solvents, such as ethanol, water, and agro-solvents. Additionally, the step of solvent evaporation prior to a fractionation procedure

was avoided. An anion exchange column chromatography was implemented in the fractionation method due to the anionic structure of rhinacanthin-C. Undesirable compounds that are non-ionic or cationic will be excluded from the column during the washing process. Subsequently, rhinacanthins were eluted by acidic ethanol to obtain RRE.

RRE was standardized to contain a total rhinacanthin content of not less than 70% w/w and not less than 60% w/w rhinacanthin-C. An RRE containing 73.7% w/w total rhinacanthins and 62.2% w/w rhinacanthin-C has been demonstrated to exhibit the same α -glucosidase inhibitory, glucose uptake stimulatory, and antiadipogenic properties as rhinacanthin-C (Shah *et al.*, 2017b). Additionally, RRE showed potent superoxide scavenging and antiglycation properties (Shah *et al.*, 2017c). RRE was also investigated for its anti-hyperglycemic and anti-hyperlipidemic activities using nicotinamide-streptozotocin-induced diabetic rats (Shah *et al.*, 2019). It was found that RRE (15 mg/kg) dramatically reduced fasting blood glucose and HbA1c while increasing insulin levels in normal rats. Moreover, RRE has been shown to normalize lipid, liver, and renal blood profiles. Furthermore, after RRE treatment, diabetic rats' islets of Langerhans were recovered. RRE (24 mg/kg/day) also showed protective effects against diabetic nephropathy in streptozotocin-nicotinamide-induced diabetic nephropathy rats through decreasing renal index, oxidative stress biomarkers, and pro-inflammatory cytokines, while enhancing renal glutathione, superoxide dismutase, and catalase (Zhao *et al.*, 2019). The anti-diabetic properties of RRE were equivalent to those of rhinacanthin-C. This observation also agrees with the antimicrobial effects of RRE (Puttarak *et al.*, 2010).

Table 1. Pharmacological activities of rhinacanthin-C

Activity	Mechanism of action	Assay	Dose or IC ₅₀
Antidiabetic	Hypoglycemic & hypolipidemic actions	<i>In vivo</i>	15-20 mg/kg (Adam <i>et al.</i> , 2016; Shah <i>et al.</i> , 2019)
	Inhibiting glucose uptake & anti-adipogenic action	<i>In vitro</i>	20 µg/mL (Shah <i>et al.</i> , 2017a)
	α-glucosidase inhibition	<i>In vitro</i>	IC ₅₀ of 22.6 µg/mL (Shah <i>et al.</i> , 2017b)
Diabetic complication	Nephroprotective action	<i>In vivo</i>	15-20 mg/kg (Zhao <i>et al.</i> , 2019)
	Anti-glycation	<i>In vitro</i>	IC ₅₀ of 8.0 µg/mL (Zhao <i>et al.</i> , 2019)
Hepatoprotection	Inhibiting fatty liver formation	<i>In vivo</i>	20 mg/kg (Gong <i>et al.</i> , 2023)
	Anti-cholangiocarcinoma	<i>In vitro</i>	IC ₅₀ of 1.5 µM (Boueroy <i>et al.</i> , 2018)
Anticancer	Anti-breast cancer	<i>In vitro</i>	3-28 µM (Chaisit and Jianmongkol, 2021), 0.1 µM (Chaisit and Jianmongkol, 2022)
Anti-inflammation	Edema and granuloma inhibition	<i>In vivo</i>	80 mg/kg (Bhusal <i>et al.</i> , 2014)
Anti-Parkinson	Motor symptom improvement	<i>In vivo</i>	5-20 mg/kg (Saleem <i>et al.</i> , 2021)
Anti-bone resorption	Inhibiting osteoclast formation	<i>In vitro</i>	0.5-2.5 µM
		<i>In vivo</i>	2 mg/kg (Tomomura <i>et al.</i> , 2015)
Neuroprotection	Inhibiting glutamate-induced neurotoxicity	<i>In vitro</i>	0.3-10 µM (Rakkhittawattana <i>et al.</i> , 2022)
	Inhibiting neuro-inflammation	<i>In vitro</i>	0.12-1 µM (Chuang <i>et al.</i> , 2017)
	Preventing subarachnoid hemorrhage	<i>In vitro</i>	400 µmol/kg (Chang <i>et al.</i> , 2016)
Immunomodulation	Inhibiting phagocytosis	<i>In vitro</i>	6.25 µg/mL (Chaisit and Jianmongkol, 2021)

As a result, RRE has been found to exhibit some biological activities similar to rhinacanthin-C. RRE is therefore an appropriate *R. nasutus* extract for the production of pharmaceutical preparations for anti-diabetic products. The extracts utilized in future research and applications should be standardized to a total rhinacanthin content of not less than 70% w/w and not less than 60% w/w rhinacanthin-C based on the adequate biological activity of RRE.

Pharmacological Activities

Although various medical applications of *R. nasutus* have been documented, rhinacanthin-C has been identified as its major active ingredient due to its reportedly diverse pharmacological activities. Table 1 illustrates the recently reported pharmacological activities of rhinacanthin-C, which include anti-bone resorption, anticancer, antidiabetic, anti-inflammatory, anti-Parkinson, hepatoprotective, immunomodulation, and neuroprotection, which some of their mechanisms of action are described as follows:

Anti-bone Resorption Activity

Many bone-related diseases, including osteoporosis and rheumatoid arthritis, are usually caused by bone resorption. Osteoclasts, or bone-breaking cells, play an important role in this process. Receptor activator of nuclear factor-κB ligand

(RANKL) is an important protein that promotes osteoclast development and activation.

Rhinacanthin-C reportedly decreased bone resorption by inhibiting the formation of osteoclasts. The molecular mechanisms of its anti-osteoclastogenic effect involved the inhibition of RANKL-induced tumor necrosis factor receptor-associated factor 6 and transforming growth factor beta-activated kinase 1 (TRAF6-TAK1) association as well as activation of MAPKs/NF-κB (mitogen-activated protein kinases/nuclear transcription factor kappa-B), which led to suppression of c-Fos (proto-oncogene) and nuclear factor of activated T cells 1 (NFATc1) that regulate transcription of genes associated with osteoclast differentiation.

Rhinacanthin-C inhibited RANKL-induced osteoclast development and bone resorption in rat models (Tomomura *et al.*, 2015). Furthermore, rhinacanthin-C demonstrated anti-inflammatory properties, suggesting its potential use in treating abnormal bone resorption due to inflammation.

Anticancer Activity

Chemotherapy is an important therapeutic option for various cancer diseases. However, cancer cells can acquire drug resistance through a variety of cellular defense mechanisms. Chemotherapeutic failure is often due to transporter-mediated multidrug resistance. This can lead to higher doses of drugs being needed, which can result in increased toxicity and an unsuccessful outcome. Multidrug resistance (MDR) in cancer patients is primarily

induced by the overexpression of drug efflux transporters, such as P-glycoprotein (P-gp). These proteins aggressively release their drug substrates from cells, thus reducing the efficacy of treatment. Suppressing P-gp function in cancer cells could avoid drug tolerance and improve chemotherapy effectiveness. Downregulation of P-gp mRNA in cancer cells may be a promising strategy for reversing MDR to chemotherapy. In addition, targeting MAPK and the phosphoinositide-3-kinase-protein kinase B/Akt (PI3K/Akt) signaling pathways can improve the efficacy of conventional chemotherapeutic treatments.

Recently, it was disclosed that rhinacanthin-C enhanced the cytotoxic effects of doxorubicin in human breast cancer cells (MCF-7) and doxorubicin-resistant breast cancer cells (MCF-7/DOX). This enhancement was achieved by inhibiting the functions of multidrug resistance-associated protein 2 (MRP2) and P-gp (Chaisit *et al.*, 2017). This implies that rhinacanthin-C may have the capability to enhance the sensitivity of human breast cancer cells to doxorubicin treatment, therefore, indicating its potential application as a chemosensitizer in cancer therapy. Another thing that rhinacanthin-C could do was lower the expression of P-gp in MCF-7/DOX cells by blocking the Akt/NF- α signaling pathway and downregulating Y-box binding protein-1 (YB-1) expression (Chaisit and Jianmongkol, 2022). In the context of human breast cancer, YB-1 is closely linked to rapidly proliferating tumors. Consequently, the inhibition of YB-1 expression leads to a decrease in the growth rate of breast cancer cells. Additionally, rhinacanthin-C demonstrated the ability to induce apoptosis in MCF-7/DOX cells through mechanisms involving heightened intracellular reactive oxygen species (ROS) levels and the suppression of cell survivals mediated by MAPKs and glycogen synthase kinase 3 β modulates the nuclear factor erythroid 2-related factor 2 (Akt/GSK-3 β /Nrf2) signaling pathways (Chaisit and Jianmongkol, 2021).

In metastatic cancer like cholangiocarcinoma, which is a life-threatening bile duct cancer metastatic liver. Cholangiocarcinoma cell invasion is typically promoted by the breakdown of the extracellular matrix via proteolytic enzymes such as matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), which are highly expressed in different kinds of tumors and help cancer invade and metastasize. Inhibition of the MAPK networks resulted in suppression of tumor growth, angiogenesis, invasion, and metastasis. Rhinacanthin-C has been reported to suppress MMP-2 protein expression in human cholangiocarcinoma cells and decrease the viability

of cholangiocarcinoma cells in a time- and dose-dependent manner (Boueroy *et al.*, 2018). Thus, rhinacanthin-C may have therapeutic potential in the treatment of cholangiocarcinoma.

Antidiabetic Activity

Type 2 diabetes mellitus (T2DM) is a major worldwide health issue that is defined by a significant increase in blood sugar levels (hyperglycemia) as well as some severe complications, such as hyperlipidemia, nephropathy, neuropathy, and cardiovascular disorders. Diabetes is characterized by hyperglycemia due to a deficiency of insulin secretion, defective insulin action, or both (Suryasa *et al.*, 2021).

In diabetic rats, the administration of rhinacanthin-C has been shown to have significant effects in decreasing fasting blood glucose, hemoglobin A1C (HbA1c), and lipid profile levels, while concurrently increasing serum insulin and the levels of pancreatic antioxidant enzymes. Furthermore, rhinacanthin-C mitigated pancreatic histopathological changes and lowered the levels of tumor necrosis factor alpha (TNF- α), inhibitor of nuclear factor-k β kinase (Ikk β), and cysteine-aspartic acid protease-3 (caspase-3) in diabetic rats (Adam *et al.*, 2016). Rhinacanthin-C was coupled molecularly with superoxide dismutase and glutathione peroxidase, which led to resolved blood lipid, liver, and kidney profiles. Furthermore, rhinacanthin-C recovered diabetic pancreas intracellularly through islets of Langerhans, a pancreatic cell that regulates blood sugar levels (Adam *et al.*, 2016; Shah *et al.*, 2019). Therefore, rhinacanthin-C may have potential applications in the treatment of pancreatic and other diabetic complications.

Diabetes nephropathy (DN) is a severe complication that affects more than 40% of people and is a leading cause of end-stage renal failure worldwide. Scarring of the glomerulus, albuminuria, tubulointerstitial renal fibrosis, and a decrease in kidney function represents all functional and structural abnormalities attributed to DN. Treatments that improve the antioxidant defense system and inhibit the release of pro-inflammatory mediators may be useful in managing or avoiding diabetic kidney failure. Diabetes-related kidney injury was alleviated by rhinacanthin-C through reducing oxidative stress and inflammation. It also boosted antioxidant enzyme amounts in the kidney (Zhao *et al.*, 2019). Thus, rhinacanthin-C might also be used as a medicinal or dietary supplement to help avoid DN.

Oxidative stress and hyperglycemia are key factors that intensify diabetic complications by accelerating the formation of advanced glycation

end products (AGEPs). In which, the overproduction of free radicals can be reduced by antioxidants. Diabetic complications are mostly caused by nonenzymatic glycation reactions between proteins and reducing sugars in chronic hyperglycemic conditions. Plant extracts and isolated phytochemicals have emerged as promising alternatives to conventional drugs for inhibiting the formation of AGEPs. They have also demonstrated potential as antidiabetic agents by means of their antioxidant properties and ability to combat AGEPs.

Interestingly, rhinacanthin-C has been reported to possess substantial abilities in scavenging superoxide and impeding albumin glycation. These findings underscore its potential utility in addressing a range of chronic conditions, with a particular emphasis on diabetes. According to kinetic analyses, rhinacanthin-C displayed potent antioxidant activity via the ErCi (electrochemical) mechanism, with an IC_{50} value of $8.0 \mu\text{g/mL}$, an antioxidant capacity of 39439 M^{-1} , and a binding constant of 45709 M^{-1} . Rhinacanthin-C exhibited glycation inhibitory activity, with an IC_{50} values of $37.3 \mu\text{g/mL}$, which could be explained by the fact that rhinacanthin-C tends to bind and mask various sites of albumin residue (Shah *et al.*, 2017c). Therefore, as a result, rhinacanthin-C has demonstrated its therapeutic utility in different chronic diseases, particularly diabetes complications.

Commercially available α -glucosidase inhibitors like acarbose, voglibose, and miglitol are expensive, have gastrointestinal side effects, and increase cardiac risks. However, inhibiting α -glucosidase is an effective strategy for controlling postprandial glucose in type 2 diabetes. Rhinacanthin-C inhibited α -glucosidase in a noncompetitive manner, while low-concentration combinations of both rhinacanthin-C and acarbose inhibited α -glucosidase in a synergistic manner (Shah *et al.*, 2017b). Therefore, rhinacanthin-C is recommended to be used as an α -glucosidase inhibitor in combination with acarbose, implying that it could be used clinically to decrease the dose and side effects associated with acarbose treatment.

Furthermore, rhinacanthin-C has been demonstrated to have a significant ability to stimulate glucose uptake, particularly in muscle cells, indicating its potential in obesity management. In adipocytes, it also had a strong anti-adipogenic effect (Shah *et al.*, 2017a). Controlling obesity involves two crucial strategies, including inhibiting adipogenesis and stimulating glucose uptake in adipose and muscle tissues. Therefore, rhinacanthin-C could be applied as an efficient adjunctive treatment for obesity-related T2DM.

Anti-inflammatory Activity

The anti-inflammatory and analgesic effects of rhinacanthin-C in animal models have been reported. The acetic acid-induced writhing test was comparable to that of indomethacin, while the formalin test indicated that rhinacanthin-C could be an effective painkiller. In addition, rhinacanthin-C exhibited anti-inflammatory effects via inhibition of edema and granuloma formation in carrageenan-induced edema and cotton pellet-induced granuloma in rats (Bhusal *et al.*, 2014).

Anti-Parkinson Activity

Parkinson's disease is the second most common neurodegenerative ailment, characterized by signs of decreased motor and non-motor coordination. Neurodegeneration in Parkinson's patients is caused by dopaminergic loss and oxidative stress, which is mostly caused by mitochondrial malfunction. Although contemporary Parkinson's disease treatments include levodopa, a dopamine agonist for improving motor instability, amantadine, an anticholinergic, monoamine oxidase-B inhibitor, and β -blockers, they are associated with substantial side effects such as muscular dyskinesia. As a result, research on natural products for Parkinson treatment has been challenging for disease progression and symptom relief with limited side effects.

A recent *in vivo* study indicated that rhinacanthin-C (5-20 mg/kg) improved Parkinson motor symptoms or cataleptic effects in a dose-dependent manner (Saleem *et al.*, 2021). Furthermore, rhinacanthin-C improved brain morphology by restoring dopamine, norepinephrine, and serotonin levels, a neurotransmitter related to Parkinson disorder. In Parkinson-induced blood profiles, rhinacanthin-C also recovers and optimizes levels of glutathione, catalase, and superoxide dismutase, while Parkinson-related mediators, including malondialdehyde and nitrite, free radical indicators, were significantly decreased (Saleem *et al.*, 2021). Rhinacanthin-C's anti-Parkinson potential is an anticipated approach to Parkinson's disease prevention and treatment.

Hepatoprotective Activity

Nonalcoholic fatty liver disease (NAFLD) is a liver disorder characterized by fat buildup in hepatocytes that can progress to cirrhosis and hepatocellular carcinoma. NAFLD affects roughly 1.7 billion people globally and is on the rise as a result of overweight and obesity. Although the processes underlying NAFLD are currently unknown, insulin resistance and high levels of fatty acids in the blood are critical to its development.

Rhinacanthin-C has potential against NAFLD by decreasing body mass, and the liver body coefficient considerably inhibits liver inflammation and fat accumulation and improves insulin resistance. Rhinacanthin-C also increased the production of adiponectin and adiponectin receptors in the liver while decreasing the expression of NAFLD-related factors, including monocyte chemoattractant protein-1 (MCP-1), TNF- α , and interleukin-6 (IL-6). Rhinacanthin-C alleviated insulin resistance and lipid accumulation in NAFLD via activation of the AMP-activated protein kinase, silent information regulator T1 (AMPK/SIRT1), and sterol regulatory element binding proteins, fatty acid synthase, and acetyl-CoA carboxylase (SREBP-1c/Fas/ACC) pathways (Gong *et al.*, 2023). As a result, rhinacanthin-C could protect against NAFLD formation by lowering body weight and glucose levels, improving liver physiological changes, and restoring NAFLD metabolic markers.

Immunomodulatory Activity

Monocytes, macrophages, and polymorphonuclear leukocytes are essential parts of the natural immune system that assist in the elimination of pathogens such as bacteria and fungi. The immune system maintains homeostasis and can be altered by pathogens, but innate immunity provides protection. Phagocytic cells produce reactive oxygen species in order to kill pathogens, but excessive production can cause inflammation and disease.

Rhinacanthin-C has a significant inhibitory impact on phagocytic activities, indicating that it can impair the immune system's ability to engulf and eradicate invading pathogens. Rhinacanthin-C at 100.0 and 6.25 $\mu\text{g/mL}$ substantially reduced phagocytosis by 26.4% and 30.6%, respectively (Chaisit and Jianmongkol, 2021). In addition, a dose-dependent reduction of myeloperoxidase activity by rhinacanthin-C was observed. Therefore, rhinacanthin-C could be used as an immunomodulatory agent to treat conditions where an overactive immune response is detrimental.

Neuroprotective Activity

There is an increasing prevalence of neurodegenerative diseases such as Alzheimer's, Parkinson's, multiple sclerosis, neuroinflammation, and amyotrophic lateral sclerosis, which have become significant health problems as the world's population has aged, with limited treatment options available. Oxidative stress and glutamate toxicity are linked to neurodegenerative diseases, and various forms of cell death, including apoptosis, autophagy, and necrosis, are involved.

Rhinacanthin-C exhibited neuroprotective effects in hippocampal neural cells by inhibiting

apoptosis, necrosis, and autophagy (Rakkhittawattana *et al.*, 2022). In addition, rhinacanthin-C improves and normalizes cell survival autophagy activity by increasing the expression of extracellular signal-regulated kinase (ERK), CCAAT/enhancer binding proteins homologous protein (CHOP), and light chain-3B (LC3B) proteins. Thus, rhinacanthin-C has potential as a neuroprotective agent for the treatment of neurodegenerative diseases.

Neuroinflammation is an inflammatory response that occurs within the central nervous system (CNS) and is defined by immune cell infiltration, glial cell activation, and the generation of inflammatory mediators. Microglia, the CNS's resident phagocytes, serve a vital function in neuroinflammation. Rhinacanthin-C has been shown to alleviate inflammation events by inhibiting inflammatory activities such as amyloid-peptide (A β)-induced production, lipopolysaccharide-activated nitric oxide generation, inducible nitric oxide synthase (iNOS) expression, and nuclear factor kappa B (NF- κ B) signaling (Chuang *et al.*, 2017). Rhinacanthin-C also inhibited neuroinflammation in microglia cells by decreasing NO generation and mRNA levels of iNOS, IL-1 β , chemokine ligand 2 (CCL-2), and chemokine ligand 2 (CCL-5). Rhinacanthin-C possesses neuroprotective properties, which could be used as a therapeutic target for neuroinflammation-related disorders.

Acute brain ischemia following subarachnoid hemorrhage (SAH) is a crucial neuralgic disorders. Multiple systems and processes contribute to the pathogenesis of vasospasm, but attempts to treat this disease has been unsuccessful. High-mobility group box-1 (HMGB-1) is implicated in cortical inflammation and may play a part in SAH-induced brain apoptosis. Rhinacanthin-C reduced the expression of pro-inflammatory cytokines and HMGB-1 in an early brain damage model after subarachnoid bleeding (Chang *et al.*, 2016). This suggests that rhinacanthin-C may have therapeutic benefits in decreasing neuro-inflammation and improving outcomes in SAH patients.

A Potential Use for Mosquito Control

The continuous use of synthetic pesticides in mosquito control is thought to encourage resistance and impede disease management. Pyrethroid tolerance in *Anopheles* mosquitoes, for example, is a common threat. The activity of detoxification enzymes, including the heme-containing cytochrome P450 monooxygenase (CYP), is upregulated as a synthetic pesticide tolerance mechanism. The enzymes CYP6AA3 and CYP6P7 have been identified as being implicated in pyrethroid resistance in *Anopheles minimus*.

Rhinacanthin-C reportedly inhibited CYP6AA3 and CYP6P7 enzymes in a reversible manner. The mechanisms of inhibition were time, concentration, and NADPH-dependent characteristics (Pethuan *et al.*, 2014). Furthermore, rhinacanthin-C made CYP6AA3 and CYP6P7-expressing cells more susceptible to cypermethrin neurotoxicity. As a result, it is possible that the combined inhibition effect of rhinacanthin-C on the mosquito cytochrome P450 enzyme and synergistic impact on cypermethrin cytotoxicity could be helpful for resistance approaches in mosquito vector control.

Metabolism

In the context of administering natural products alongside therapeutic agents, the study of their metabolism and pharmacokinetics plays an important role in understanding herb-drug or herb-herb interactions. Notably, the inhibition and activation of drug-metabolizing enzymes and drug transporters are key mechanisms of interference that can give rise to concerns related to both the effectiveness and safety of such combinations.

The pharmacokinetics of rhinacanthin-C are closely linked to drug transporter proteins and drug-metabolizing enzymes. It has been observed that rhinacanthin-C inhibits various transport proteins, including P-gp, MRP2, and breast cancer resistance protein (BCRP). However, these inhibitory effects were reversible. Rhinacanthin-C also inhibited drug-uptake proteins, specifically affecting organic anion-transporting polypeptide B1 (OATP1B1). Pharmacokinetic studies have indicated that rhinacanthin-C exerted an influence on Phase I drug-metabolizing enzymes, encompassing a range of human CYP isoforms, including CYP2C8, 2C9, 2C19, 3A4/5, and 3A4/5. In contrast, rhinacanthin-C had no impact on CYP1A2, 2B6, 2D6, and 2E1 isoforms (Pouyfung *et al.*, 2014; Dunkoksung *et al.*, 2019). Unfortunately, the data on the influence of rhinacanthin-C on placental CYP1 activity as well as CYP1A1 and ABCG2 expressions is limited. Rhinacanthin-C selectively and permanently inhibited CYP2A6 and 2A13 via coumarin 7-hydroxylation in a concentration, time, and NADPH-dependent manner (Wongwanakul *et al.*, 2013; Wang *et al.*, 2021). Therefore, it is important to consider herb-drug interactions when rhinacanthin-C is used in conjunction with other conventional medications.

Rhinacanthin-C pharmacokinetic and toxicity profiles were also reported utilizing computational analyses. According to Lipinski's rule, rhinacanthin-C demonstrated drug candidate behavior. Based on

ADMET simulations, rhinacanthin-C easily crosses the blood-brain barrier. Rhinacanthin-C also exhibits good oral bioavailability and intestinal absorption due to its strong inhibitory property against P-gp. Recently, the *hERG* gene has been investigated as a potential target in preclinical drug development. In this regard, rhinacanthin-C has a low affinity for inhibition of *hERG*, which increases its druggability. Furthermore, the ADMET results indicated rhinacanthin-C affinity as a CYP450 3A4 substrate, and its terminal methyl group and aromatic portion appear to be vulnerable to oxidation (Zhao *et al.*, 2019).

Although *in vitro*, *in vivo*, and *in silico* experiments may indicate that rhinacanthin-C is safe for various treatments, clinical trials are still required to confirm its safety for human use.

Toxicity Assessment

Although natural products are generally believed to be less hazardous than synthetic products, a rising number of case reports reveal acute or chronic intoxications caused by natural product uses. As a result, assessing the toxicity of natural products is critical to ensuring their safety before they are used as an alternative treatment. Prior to clinical use, animal and non-animal models can be used to assess toxicity. Animal models are regarded as the gold standard in toxicological studies since the entire animal is often closely comparable to human toxicity and, more significantly, includes pharmacokinetic and ADME (absorption, distribution, metabolism, and excretion) profiles. Nonetheless, the use of *in silico* modeling in toxicity assessment is a potential endeavor in innovative drug discovery since it typically speeds up the process and reduces costs.

Up to date, no conclusive evidence of rhinacanthin-C toxicity assessment has been established. However, some toxicity studies of *R. nasutus* extracts and rhinacanthin-C have been documented. An *in vitro* toxicity investigation of a chloroform extract of *R. nasutus* roots in the Raji cells, a lymphoblast-like cell line, revealed no significant harm (Murakami *et al.*, 1993). In addition, rhinacanthin-C (0.125 μ M) exhibited non-neurotoxic damage to rat hippocampus neurons (Chuang *et al.*, 2017). Furthermore, an *in vivo* toxicity investigation of ethanol and water extracts of *R. nasutus* (10 μ g/kg) in mice revealed no significant toxicity after subcutaneous injection and feeding of the extracts containing rhinacanthin-C through a gastric tube. Another *in vivo* study on the chemoprotective impact of rhinacanthin-C found that rhinacanthin-C (25-50 mg/kg/day) had no effect

on aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels in mice. However, it has been suggested that extended and excessive usage of rhinacanthin-C should be avoided in patients with hepatic problems (Siripong *et al.*, 2010).

Based on an *in silico* experiment, rhinacanthin-C achieved a toxicity profile that was non-toxic, non-lethal, and ranged in category III of acute oral toxicity (LD₅₀ values of 500-5,000 mg/kg), which is more than 10 times greater than the therapeutic dose of rhinacanthin-C (Zhao *et al.*, 2019). Although rhinacanthin-C has demonstrated diverse cytotoxicity activities, the majority of them were specific to tumor or parasite cells. Continuous administration of rhinacanthin-C, on the other hand, may overcome the unique bioactivity of rhinacanthin-C and be toxic to normal cells.

Future Perspective and Conclusions

Rhinacanthin-C is an important biologically active substance obtained from *R. nasutus* leaves and roots. It possesses various pharmacological activities that benefit therapeutic applications for a number of chronic diseases, including diabetes, cirrhosis, breast cancer, inflammation, osteoporosis, neurodegenerative, and immune deficiency diseases. The present review also illustrates some safety and toxicity information to confirm the druggability of rhinacanthin-C. However, the majority of research documents stem from *in vitro*, *in vivo*, and *in silico* studies. Therefore, further clinical research on rhinacanthin-C is required to assess its potential as a therapeutic agent. An alternative approach to developing rhinacanthin-C based herbal products may involve the use of RRE, which offers some comparable pharmacological benefits, such as antidiabetic and antibacterial effects, with a low-cost of production. However, it is essential to thoroughly evaluate the equivalent therapeutic effects of RRE with rhinacanthin-C. Such research would ensure that the use of RRE not only maintains the desired pharmacological properties but also enhances the economic feasibility of herbal product development.

Abbreviations

ADME	Absorption, distribution, metabolism and excretion
AGEPS	Advanced glycation end products
ALT	Alanine aminotransferase
AMPK/SIRT1	AMP-activated protein kinase; silent information regulator T1

AST	Aspartate aminotransferase
BCRP	Breast cancer resistance protein
Caspase-3	Cysteine-aspartic acid protease-3
CCL	Chemokine ligand
CHOP	CCAAT/enhancer binding proteins homologous protein
CYP	Cytochrome P450
DMAPP	Dimethylallyl pyrophosphate
EIMS	Electron ionization mass spectroscopy
ERK	Extracellular signal-regulated kinase
ErCi	Electrochemical
GPP	Geranyl pyrophosphate
GSK-3β/Nrf2	Glycogen synthase kinase 3β modulates the nuclear factor erythroid 2-related factor 2
HbA1c	Hemoglobin A1C
HMGB-1	High-mobility group box-1
IL	Interleukin
Ikkβ	Inhibitor of nuclear factor-κβ kinase
iNOS	Oxide synthase
IPP	Isopentenyl pyrophosphate
LC3B	Light chain-3B
MAPKs/NF-B	Mitogen-activated protein kinases/nuclear transcription factor kappa-B
MCF-7/DOX	Doxorubicin-resistant human breast cancer cells
MCP-1	Monocyte chemoattractant protein-1
MDR	Multidrug resistance
MMP	Matrix metalloproteinase
MRP2	Multidrug resistance-associated protein 2
NAFLD	Nonalcoholic fatty liver disease
NF	Nuclear factor
NFATc1	Nuclear factor of activated T cells
OATP1B1	Organic anion-transporting polypeptide B1
OSB	O-succinylbenzoate
P-gp	P-glycoprotein
PI3K/Akt	Phosphoinositide-3-kinase-protein kinase B/Akt
RANKL	Receptor activator of nuclear factor-Β ligand
RRE	Rhinacanthins-rich extract
ROS	Reactive oxygen species
SAH	Subarachnoid hemorrhage
SREBP-1c/Fas/ACC	Sterol regulatory element binding proteins; fatty acid synthase; acetyl-CoA carboxylase
TAK1	Transforming growth factor beta-activated kinase 1

TNF- α	Tumor necrosis factor alpha
TRAF6	Tumor necrosis factor receptor-associated factor 6
YB-1	Y-box binding protein-1 enriched extract

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