Potential cardio-protective agents: A Resveratrol review (2000-2019)

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Abstract

With a 2030 projection of 23.6 million deaths per year, the prevalence and severity of cardiovascular disease are astoundingly high. Thus, there is a definitive need for the identification of novel compounds with the potential to prevent or treat the disease and associated states. Moreover, there is also an ever-increasing need for drug delivery systems (DDS) that cope with poor and ranging physiochemical properties of therapeutic compounds to achieve the clinical effect. The usage of resveratrol (RES) is a growing area of interest with innumerate pieces of research, evidencing the drug's efficacy. This drug is, however, marred; its notably poor physiochemical properties (namely poor water solubility) limit its use for oral drug delivery. RES analogues, however, potentially possess superior physiochemical characteristics offering a remedy for the aforementioned drawback. However, particulate based DDS are equally able to offer property amelioration and targeting. This review offers an extensive examination into the role of RES as a potential cardioprotective agent. The prevalence and suitability of associated analogues and the role of nanotechnology in overcoming physicochemical boundaries, particularly through the development of nanoparticulate formulations, will be discussed in detail.

KeyWords

Resveratrol, cardiovascular disease, bioavailability, nanotechnology.

1. INTRODUCTION

According to the world health organisation (WHO), cardiovascular diseases (CVDs) (ischemic heart disease, hypertension, heart failure, *etc.*) account for ~50% of non-communicable diseases (NCDs) worldwide and are responsible independently for 17.3 million deaths per year, with projections estimating ~23.6 million by 2030 [1-4].

Predicted economic losses, in the period of 2011 to 2025, in low and middle-income countries (LMICs), which account for 80% of deaths associated with NCD [5]. By reducing CVD mortality rates alone by as little as 10%, a reduction of ~$377 billion in economic losses during this period is deemed possible [6]. Investing in CVD prevention/treatment is therefore paramount, with economists suggesting over a 6-fold increase in losses (amounting to as much as $47 trillion worldwide) if this remains unaddressed 25 years from now. Currently, the prescribed WHO "best buy" interventions render this potential loss avoidable, as it projects costs of 11 to 13 $billion annually.

In response to the gravity of this matter, there have been a number of recent advancements in technological and pharmaceutical therapies, which have been successfully implemented in managing CVDs, decreasing their mortality rate [7]. Various heart failure (HF) treatments, which include pharmacological interventions (drug therapy), the usage of cardiac defibrillators, and also heart transplantation, have been successful in reducing the mortality from heart failure [8-11]. Despite this, the mortality rate from HF remains relatively high. Consequently, novel therapies that focus on improving HF patient's health outcomes and quality of life are being developed [12].

Alternate therapies have employed nutraceuticals to provide physiological benefit in the treatment of chronic conditions, which additionally target various critical factors, including: oxidative trauma [13], inflammatory responses [14], deprived endothelial function [15] in CVD and HF progression [16]. One such natural compound which has been substantially investigated and considered for its potential in HF and CVD treatment/prevention is resveratrol (3,5,4'-trihydroxystilbene) (RES).

RES belongs to a group of plant compounds referred to as polyphenols, exhibiting antioxidant properties [17]. The compound is present either in free form, or bound as glucosides, and exists with two isomeric structural forms, *cis* and *trans* Fig. (**1**), with the latter occurring notably more than the former, possessing the ability to isomerize to *cis*upon exposure to light or Ultraviolet (UV) radiation [18]. RES biosynthesis is a two-step reaction dependent on the stilbene synthase (STS) enzyme Fig. (**1**), which in turn is produced as a response to stress factors such as; UV radiation, injury and pathogenic infection [19, 20].

Initially identified in 1940, RES was derived from the roots of Veratrum grandiflorum, later in 1963, it was also discovered in the dried roots of Japanese knotweed (Polygonum cuspidatum) [22, 23]. RES has subsequently been utilised in traditional eastern herbal medicine, indicated as a treatment for a multitude of conditions (gonorrhea, Vasculitis?, atherosclerosis hypertension, hyperlipidemia, diabetes, dermatitis, and various cancers,) [24].

The definitive lack of any severe side effects has prompted significant interest in this compound [24, 25]. To date, approximately 70 plant species have been identified to contain RES, with grapes identified as a crucial source (50 - 100 µg/g in skin and seeds) [26].

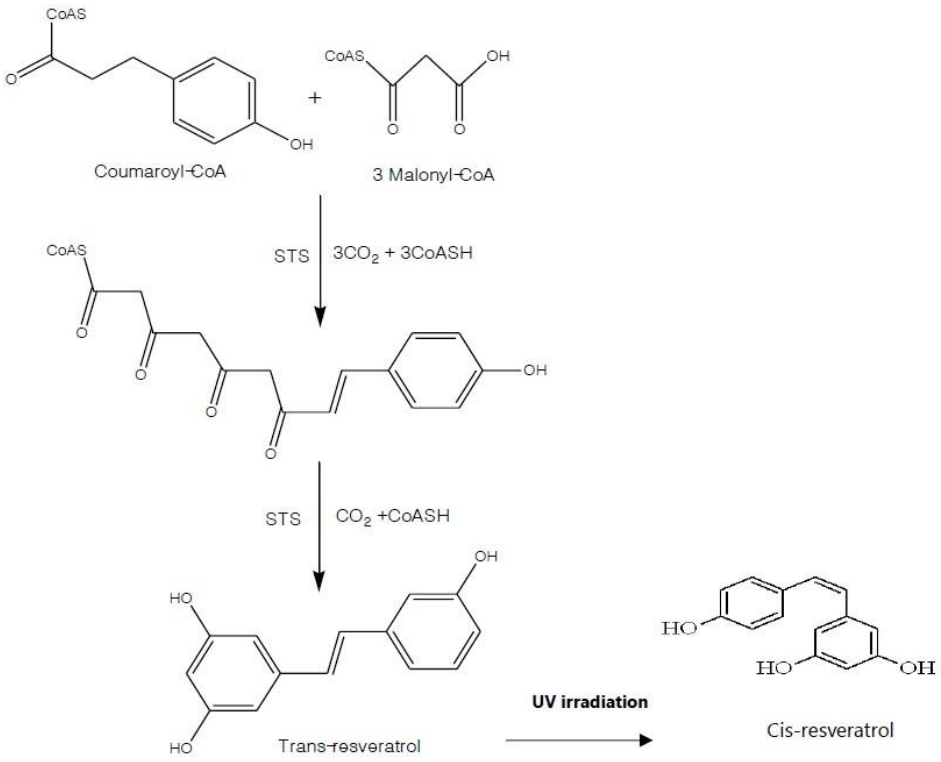

Fig. (1). Resveratrol biosynthesis and its structural isomers: (A) cisisomer (B) trans-isomer. STS is involved in the catalysis of three condensation reactions between coumaroyl-coenzyme A (CoA) and three molecules of malonyl-CoA through cleavage of three carbon dioxide molecules. In the second step the terminal carboxyl group lost is, which leads to the production of the C14 molecule resveratrol [20, 21].

2. ABSORPTION, METABOLISM AND BIOAVAILABILITY OF RES

A significant drawback of Resveratrol is its circumscribed bioavailability; this impacts negatively upon its clinical usage of in the treatment of cancerous diseases. Absorbed from the gastrointestinal tract, resveratrol is rapidly metabolised into its glucuronides, sulfates and hydroxylates (Figure 2). Two hours post ingestion, the resveratrol metabolites 3- and 4'-O-sulfate, and 3-O-glucuronide conjugates can be detected [27]. Intestinal bacteria are believed to be involved in this metabolic process, with the observed fractional ratio of metabolites varying amongst individuals. Research conducted by Park *et al.*, (2001) has demonstrated the conversion of resveratrol by gut bacteria into metabolites; dihydroresveratrol, 3,4'-dihydroxy-transstilbene and 3,4'-dihydroxybibenzyl [28]. The rapid metabolism of resveratrol in healthy volunteers irrespective of the number of doses administered (single or multiple) provides a narrow window of opportunity for resveratrol to treat cancerous cells, regardless of dose. Consequently, research has been focused on methods by which metabolism of the compound may be slowed, with the development of synthetic analogues of the compound successfully impeding the compounds swift metabolism [29, 30].

The by-products of the metabolism of resveratrol have been posed to possess biological activity. Specifically, the metabolite resveratrol 3-sulphate has been suggested to possess chemo-preventative properties. Previous research has determined the body's ability to absorb both the compound and its metabolites at various points in the gastrointestinal tract, with concentrations of both detectable at a tissue level. Interestingly, these metabolites further possess the ability to convert back into the original resveratrol molecule. This impact of this ability is enhanced permeation of resveratrol and its metabolites into target tissues, potentially increasing anti-cancer action [29].

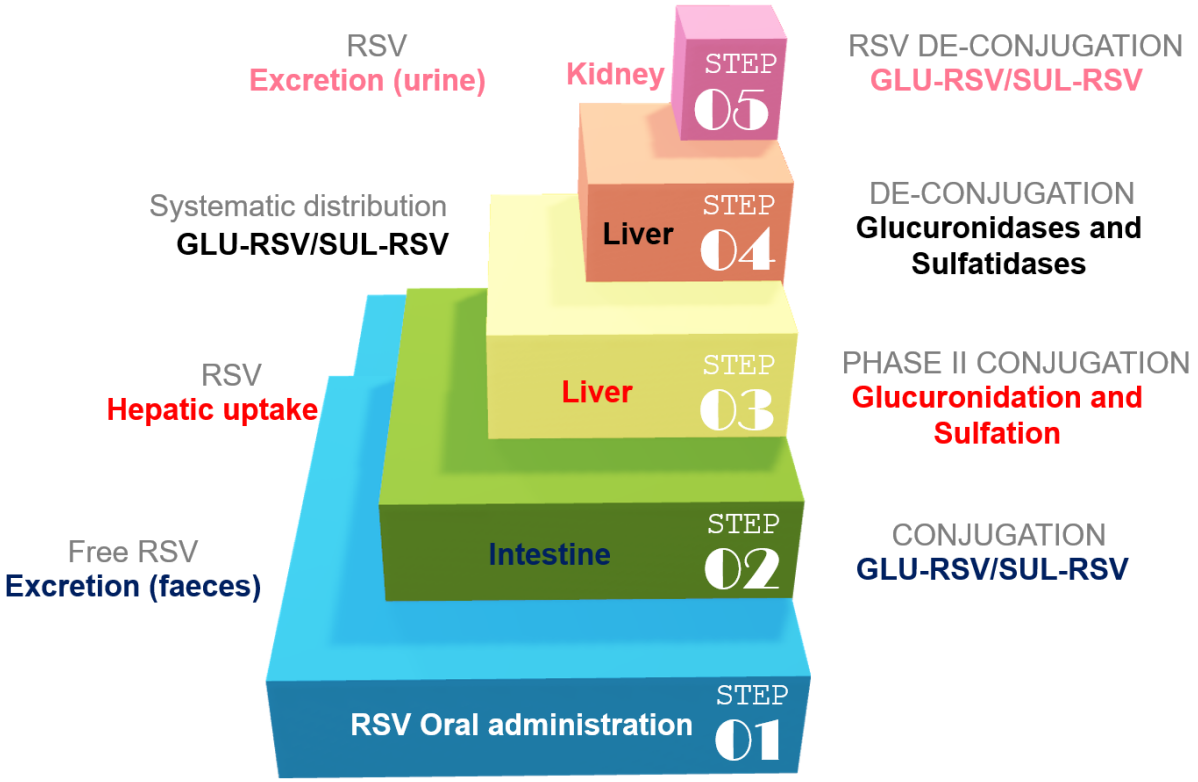

Fig. (2). RES metabolic pathways; the 5 steps from oral administration to final two metabolites excreted in urine.

3. MEDICAL BENEFITS OF RES AGAINST CVD

RES is a stilbene derivative and is a phytoalexin of plant origins [22]. It is abundant in grapes, and subsequently, red wine, with plants containing the compound used extensively as a potent antioxidant and in the treatment of various disease states Fig. (**3**) [32, 33]. This accounts for the compound's association with the "French paradox", *i.e.*, the reasonable consumption of red wine reduces the incidence of CVDs among the French population, despite diets rich in fat [34]. A plethora of polyphenols, as opposed to RES alone, may also account for this trend; however, RES remains a strong potential candidate, owing to its cardio-protective properties (*e.g.,* Preventing vasculitis, oxidation, thrombus formation) [35,

36]. Results from high dosage supplements commonly used in research compared to lower concentrations observed in wine are fraught with difficulty due to the large concentration difference [37]. Additionally, in spite of a surge in published studies on RES, a minuscule number relates to clinical experimental trials [38]. Thus, longitudinal controlled clinical trials are crucial in confirming the proposed beneficial cardiovascular effects.

There are, however, a number of preclinical studies that have been carried out using animal models illustrating the potential beneficial effects of RES upon CVDs [40]. A number of molecular targets have been recognised for RES, including the silent information regulator 2/sirtuin 1 (SIRT- 1), AMP-activated protein kinase (AMPK), nuclear factors (erythroid-derived 2)-like 2 (Nrf2) and nuclear factor-kappa B (NF-B). RES is noted to stimulate the production of nitric oxide in the endothelium, this in turn, diminishes oxidative strain and impedes vascular inflammation Preventing clots [39].

Upon examination of animal models with cardiovascular disease, RES has been noted to protect the heart from ischemic damage, reducing blood pressure, and slowing the development of atherosclerosis [39]. A wide variety of targeting agents have been identified to mediate the aforementioned cardiovascular effects of RES; amongst these are the oestrogen receptor α, adenosine receptors, cyclooxygenase 1, histone/protein deacetylase sirtuin 1, AMP-activated protein kinase, Akt kinase, nuclear factor-E2-related factor-2, and NF-κB [41].

The identified benefits of using resveratrol in terms of atherosclerosis, hypertension, stoke, myocardial ischemia and heart failure through clinical studies have raised various discussions. Additionally, recent research has demonstrated that pretreatment with RES restores heart tissue antioxidant levels through a reduction in the infarct size and necro-enzyme level [42-44].

Contrastingly, present pharmacotherapies focus heavily upon correcting neurohumoral factors, which are known to have an effect on CVD and heart failure (HF) [8]. Alternative therapies, which utilize nutraceuticals that directly target alternative factors involved in CVD and HF development are also an avenue of interest for the prevention and treatment of both the aforementioned disease states [16, 45].

In preclinical studies, compounds of natural origin have been demonstrated to be effective in the treatment of underlying causes of CVD and HF, including oxidative stress [13], inflammation [14], and deprived endothelial function [15], in addition to poor left ventricle function [46].

Consequently, nutraceuticals may offer to target specific features of CVD and HF, which are not effectively managed or treated with current pharmacotherapies. Whilst identified for its potential independent use, nutraceuticals are also considered for potential use as addon/supplementary treatments to mainstay therapies for HF. Currently, whilst in initial stage trials, these natural compounds form the basis of synthetic analogues with enhanced efficacy [22]. RES is an example of one such compound offering scope to help in the treatment of HF and CVD. This article aims to examine the evidence pertaining to the clinical utilisation of RES in the treatment of CVD and HF treatment [39].

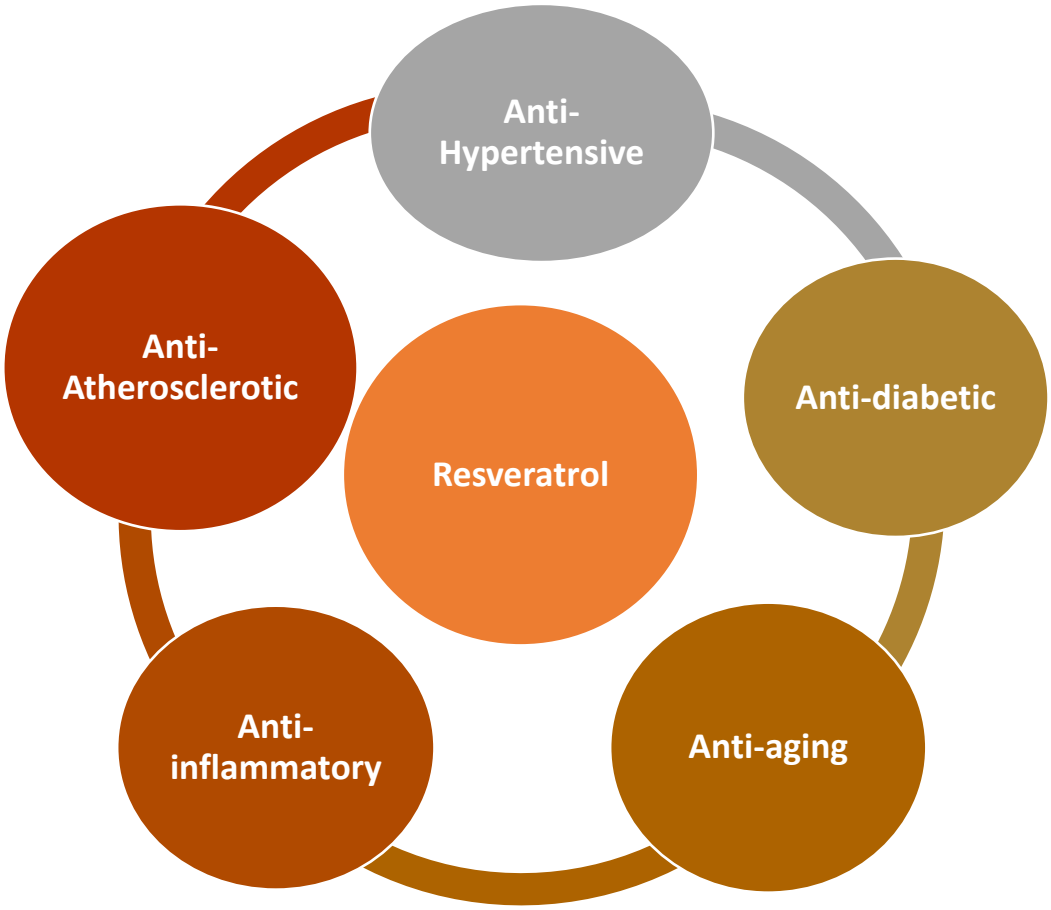

Fig. (3). Medical benefits of RES. RES metabolic pathways; the 5 steps from oral administration to final two metabolites excreted in urine.

4. ANTI-ATHEROSCLEROTIC EFFECTS OF RES

Atherosclerosis Atherosclerosis is a condition which primarily influences the inner coat layer of blood vessel walls. It is characterised by the retention of lipids in the extracellular compartments, in addition to the production of an inflammatory response. The aforementioned processes result in the narrowing of the vessel lumen and/or thrombus formation, which may bring about clinical events (*e.g.,* stroke and coronary and peripheral artery disease). Amelioration of the lipid profile of individuals is an area of interest, owing to their involvement in the atherosclerotic process, particularly low-density lipoproteins (LDL). Preclinical studies have demonstrated RES to possess potential in modifying lipid profiles by reducing plasma triglycerides and LDL cholesterol levels, whilst raising (High-density lipoprotein) HDL-cholesterol [47]. As a supplementary addition, research conducted by Cho *et al.* 2008 indicated that RES possessed the ability to potentiate hypocholesterolemic action of lipid regulating drugs such as pravastatin; through down-regulating the 3-hydroxy-3-methyl-glutaryl-CoAreductase (HMG-CoA reductase), an enzyme which is responsible for intervening in the initial stages of cholesterol biosynthesis [48]. Additionally, RES has been suggested to enhance the expression of LDL receptors suggesting additional mechanisms of action in reducing blood LDL-cholesterol concentrations [49]. Moreover, RES is also proposed to be a powerful antioxidant, potentially reducing LDL oxidation, a process that plays a direct role in atherogenesis (clot formation mechanism). RES is also believed to impact CVD by inhibiting the smooth muscle cell movement, which additionally decreases circulating LDLcholesterol levels, further reducing CVD risk. There are a number of additional mechanisms by which RES is believed to reduce the risk of CVD irrespective of lipid profile, including prevention of lipid oxidation, platelet aggregation, arterial vasodilation and modulation of lipoproteins. Furthermore, the anti-oxidative action of RES is thought to further reduce oxidative stress and bring about the regeneration of alpha-tocopherol, which further reinforces antioxidant defence mechanisms. In terms of the compound's safety profile, RES has demonstrated no significant toxicity even when exposed to elevated concentrations. Thus, RES is suggested as an effective anti-atherogenic compound, which may be utilised in the prevention and treatment of CVD [50].

5. ANTI-HYPERTENSIVE EFFECTS OF RES

Hypertension Hypertension (high blood pressure) remains a chief contributing factor to the development of CVD [51]. The proposed mechanisms by which RES is believed to lower blood pressure based upon preclinical experiments include: increase in endothelial NO pro duction, the elevated expression of SIRT1 in the endothelial cells, reduces the inflammation, oxidative stress and Ca2+ entrance into the cells [37].

The usage of several animal models has been instrumental in demonstrating the anti-hypertensive effects of RES, when treatment with 10-320 mg/kg was administered daily for a period ranging from 14 days to 10 weeks [37]. This reduction in the blood pressure was also achieved notably in insulin-resistant animal models when a low dose of 5 mg/kg/day was administrated, indicating the suitability of RES in the treatment of hypertensive patients with co-morbidities such as diabetes or metabolic syndrome [52]. In a number of research publications, RES has been demonstrated to reduce blood pressure, *e.g.,* Dolinsky et al. 2011 noted in two hypertensive animal models that an elevated dose of RES alleviated hypertension and negated the development of cardiac hypertrophy [40].

RES also demonstrated efficacy in reversing cardiac hypertrophy and contractile dysfunction [53]. Recent research conducted by Thandapilly *et al.,* (2013) demonstrated RES to be effective in lowering the blood pressure in 28 week old naturally hypertensive rats; however when combined with the anti-hypertensive hydralazine (diuretic drug), the effect was more pronounced when using the anti-hypertensive agent alone in improving cardiovascular parameters [54]. Previous studies are in agreement upon the mechanism by which RES brings about a drop in blood pressure, when RES therapy was administered for a short period, thisis related to the mechanisms involved upon which RES reduce the blood pressure *i.e.*, endothelium-dependent, with the implication of AMPK (a regulator of energy metabolism), SIRT-1 and Nrf2 [37]. Both of these result in vasodilation through elevated exposure to nitric oxide (NO) relative to the activity of eNOS [55, 56] and the latter constitute the antioxidant activity of RES [57]. RES causes the activation of SIRT-1 thought to increase the expression and activity of eNOS [58]. RES also possesses the ability to activate AMPK, as a result increasing NO production and hence vasodilation [59]. This is corroborated by testing in

various animal models, which have demonstrated that RES improves vasodilation [60]. Endothelium-independent mechanisms have also been identified as being involved in the therapeutic effects noted for RES [37]. RES is theorised to cause inhibition of angiotensin II (AngII)-induced phosphorylation. Zordoky *et al.,* (2015) further demonstrated RES inhibition of aortic contraction caused by AngII. This is supported by RES related decrease in AngII-induced hypertensive mice when administered daily [61]. Animal model studies have provided scope for RES mediated organ protection through its anti-hypertensive action [59], which provides a foundaion in terms of potential treatment or cotreatment of hypertension, prompting further investigation into the clinical effects of RES upon blood pressure [62, 63]. Though, clinical data relate to the antihypertensive activity of RES upon blood pressure remaining inconclusive. Firstly, the reduction in blood pressure is only observed with respect to the systolic blood pressure alone [64]. It is well known that elevated systolic blood pressure is one of the main risk factors for developing CVD, as opposed to diastolic blood pressure [65]. Additionally, A study conducted by Beshay et al. commented on the difficulty of directly linking vasodilation with RES in patients with metabolic disorders [66]. Furthermore, recent research conducted by Theodotou et al. 2017 on patients with hypertension demonstrated that RES may be used as an add on treatment to angiotensin-converting enzyme (ACE) inhibitors in order to sufficiently regulate the blood pressure obviating the requirement of additional anti-hypertensive medications [67]. Endothelium-dependent vasorelaxation is also improved when exposed to RES, with research showing that early treatment with RES acts to preserve endothelial function, reduces oxidative stress, superoxide dismutase activity and progressively lowers the incidence of hypertension with a reduction in hydrogen peroxide levels [13, 59].

6. THE ANTI-DIABETIC EFFECT OF RES

Diabetes is notable comorbidity commonly associated with heart failure [68, 69], with circa 40% of patients having both disease states [68, 70]. Deteriorating diabetes has been established as a causative factor in inducing HF, independent from Coronary artery disease (CAD) and hypertension. HF is observed two to four-fold greater in terms of incidence in diabetic patients in comparison to non-diabetic patients in accordance with a recent

Framingham Heart Study [71]. It is proposed that diabetes may come first before the progression into cardiac dysfunction and HF. Moreover, there is a suggestion which proposes that HF can actually lead to the development of diabetes [72]. The existence of both diabetes and HF leads to a poorer disease prediction, poorer quality of life, hospitalisation, and finally, death in the affected populations [73]. Additionally, the presence of diabetes raises the danger of myocardial infarction (MI) in HF patients. Irregularities in the glycemic parameter are a result of the absence of insulin, this impacts cardiac activity by changing the regular myocardial functions leading to HF. Additional diabetes-related features, which influence the myocardium include lipid toxicity, oxidative stress, and inflammation [74].

At present, there is a definitive absence of clarity surrounding the accurate definition of diabetic cardiomyopathy and pathology. Clinical and pre-clinical documentation is indicative of the importance of continuing diastolic dysfunction and systolic dysfunction in the development of HF [75].

In combination with HF therapy, the management of diabetes is also believed to be of paramount importance, particularly when both are present as comorbidities. Currently, the treatment of patients with HF does not differ in the presence or absence of diabetes [71, 76]. Despite this, the development of innovative diabetic treatments, for instance: sodium-glucose cotransporter (SGLT)2 inhibitors, may offer potential in improving the prognosis for HF patients with or without diabetes [77]. In addition to its direct cardio-protective effects, RES is thought to improve insulin sensitivity in addition to glucose breakdown in rodents and primates with the following disease states; type 1 and 2 diabetes, metabolic syndrome; in addition to age-related complications [78]. These anti-diabetic properties have been associated with a reduction in glucose manufacture, initiation of the AMPK (a key controller of breakdown), enhanced glucose uptake through a rise in glucose transporters, and a decrease in oxidative stress [79]. Moreover, RES has also been demonstrated to recover cardiac assembly and function in the presence of type 1 and 2 diabetes [80]. This is consistent with pre-clinical studies, which have further supported claims of RES's anti-diabetic properties [81, 82]. A recent meta-analysis of 11 pieces of research determined short-range utilization of RES to reduce fasting glucose, insulin, glycated haemoglobin and in insulin resistance in diabetic patients [83].

7. ANTI-INFLAMMATORY EFFECTS OF RES

The connection between vascular inflammation and increased danger of cardiovascular disease, particularly hypertension and atherosclerosis, is reported [84].

During atherosclerosis, the initial phases in which atherosclerotic lesions develop are typically characterised by endothelial cells commencing expression of selective adhesion molecules, e.g. vascular cell adhesion molecule-1 (VCAM-1), which facilitate the adhesion of leukocytes to endothelial cells [83]. This process is significantly more visible in areas where the endothelium is damaged and the flow and manufacture of nitric oxide (NO) are reduced or distorted. Additionally, smooth muscle cells within the injured sections may yield proteoglycans which adhere to lipoproteins. These help in their oxidation and enhance the attachment of leukocytes to the atherosclerotic injury in the arterial vessel walls [86, 87]. Following attachment, the leukocytes (lymphocytes/monocytes) are able to access the intima and initiate a local inflammatory reaction [85]. In addition to this process, the production of factors also causes monocytes to mature into macrophage foam cells in addition to the production of inflammatory cytokines by T-cells, which support smooth muscle cells in the endothelium, ultimately forming an extracellular matrix of smooth muscle cells and fibrin [88]. Owing to the contribution of inflammation in the process of atherosclerosis, pro-inflammatory cytokines are frequently utilised as biomarkers in order to allow the potential identification of patients with heart failure, following adjuvant therapy with a potentially cardioprotective therapeutic agent [85]. There are a number of common biomarkers which are involved in the inflammatory response measured in RES clinical trials; these include but are not limited to: interleukin (IL)6, tumor necrosis factor (TNF), c-reactive protein (CRP), Intercellular Adhesion Molecule 1 (ICAM-1), P selectin, and E selectin [85]. Additional inflammatory cytokines implicated in cardiac disease states which are used in RES clinical trials in order to evaluate the risk of atherosclerosis and HF include IL-8; (a marker to monitor negative effects) and IL-10 (an anti-inflammatory agent and a marker of desirable effects) [88].

8. ANTI-AGING EFFECT OF RES

Increasing age is also a well-known factor for the development of CVD. Delaying the physical progression of aging and thus the incidence/ development of age-related chronic diseases is a current problem of today's age. RES plays a potential role in this process, with it being proposed to activate anti-aging genes and demonstrating an extension of the lifespan of simple organisms such as yeasts and worms [89]. The compound was also demonstrated to increase the life span of high-calorie-diet-fed mice, offsetting the undesirable impact of overweightness and insulin resistance (*e.g.,* slowing aging), as in normally fed mice, no significant difference in overall survival and lifespan was observed following supplementation with the cardioprotective agent [91]. These proteins (Fig. **4**) are crucial for NF-jB, p53, eNOS, and peroxisome-proliferator-activated receptor co-activator (PGC-1a) deacetylation [92], which shortens NF-jB response and inhibits p53 activity. Part of this response is believed to be mediated by RES, with the mechanism being theorized as suppression of p53, expressed as a response to a plethora of forms of stress which involve DNA impairment and, ultimately, cell death (apoptosis). Apoptosis delay provides a window for cell reparation of impairment and preventing cell apoptosis [93]. Contrastingly, in cancerous cells, RES has been found to demonstrate an anti-proliferative effect, which is substantially reinforced by the downregulation of the proteins involved in the cell cycle in addition to inducing cell apoptosis in tumorigenic cells [94].

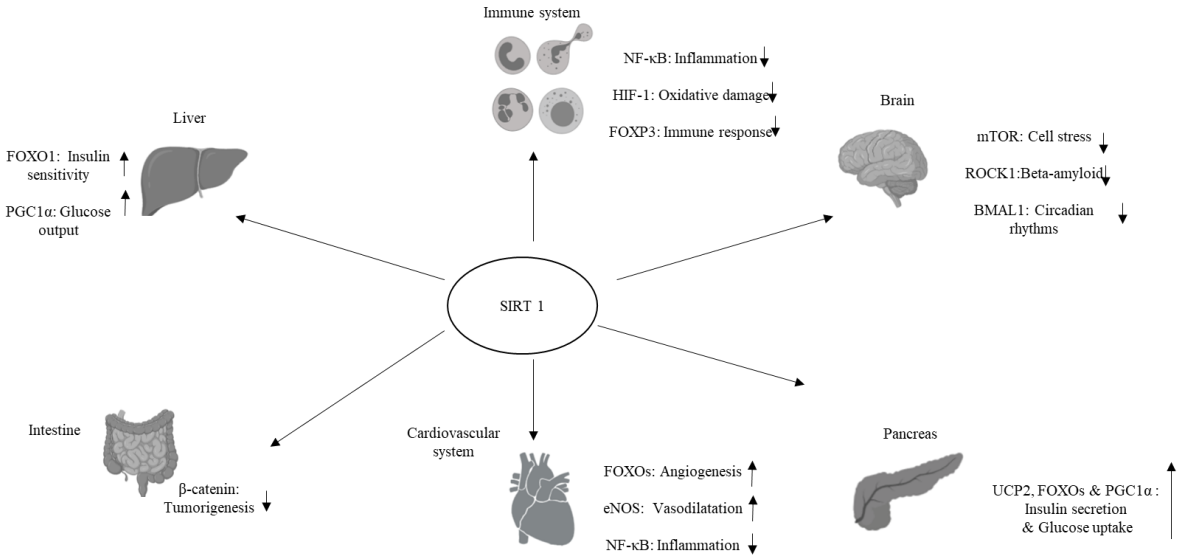

Fig. (4). Physiological functions and molecular targets of SIRT1. Adapted from [95].

9. DELIVERY CHALLENGES OF RES

Whilst there is the clinical evidence, which suggests the anti-cancer action of RES, actual clinical application is limited due to its poor physicochemical properties (low bioavailability) and prompt clearance from circulation upon administration [96]. In addition to the drug's poor bioavailability, the compound possesses poor water solubility and chemically unstable, despite high bioactivity, presenting significant challenges in terms of drug delivery. RES is prone to chemical degradation when exposed to high temperatures, pH changes, light and certain enzymes. This chemical breakdown frequently comprises the isomerisation of RES to *cis* form upon the exposure to UV light and, therefore, can lead to a reduction of biological activity after isomerisation; this contributes to bioactivity and bioavailability limitations of RES. It is also subjected to first-pass breakdown following absorption, for instance, sulfate and glucuronide conjugates are quickly produced in the body [97].

Particularly in terms of aqueous-based pharmaceuticals, the poor aqueous solubility is unfavourable for the development of high concentration formulations. Not only is this challenging from a formulation perspective, but also poor water solubility is linked to reduction in dissolution rate and limitation of cell absorption [98], ultimately resulting in reduced oral bioavailability after complete metabolism. Moreover, as a result of the poor bioavailability of the therapeutic agent, concentrations achieved at target tissues are significantly lower than required for efficacy in human subjects. Research has demonstrated that the oral absorption of RES was as minimum as of 75% and that poor bioavailability was linked to rapid and complete metabolism [99]. Additionally, the half-life of RES is of short duration, ranging between 8-14 minutes being metabolized into sulfate and glucuronide metabolites in the liver and intestinal epithelial cells in humans; this has been identified as the rate-limiting step to RES's bioavailability. In addition to the issues relating to metabolism and bioavailability, the *trans*-RES isomer of the compound is known for possessing poor photostability, being readily oxidized and demonstrating unfavorable pharmacokinetics [100]. Owing to these properties, successful clinical application for therapeutic or prophylactic purposes using RES is a challenging achievement. Methods to increase solubility and bioavailability have been examined by researchers, including; the co-administration of *trans*-RES metabolism inhibitors in addition to seeking out alternative analogs and delivery

systems [101]. The therapeutic activity of RES may only be achieved if bioavailability issues *in vivo* are addressed [102].

10. ALTERNATIVE ROUTES OF ADMINISTRATION

The low bioavailability of RES following oral administration has been proposed to be remedied *via* exploration of alternative delivery routes; particularly routes which avoid the first-pass metabolism in addition to bypassing the gastrointestinal tract, which would, in theory, raise the bioavailability of RES and subsequent concentration at targeted tissue. For example, a novel inhalable RES formulation has been identified as a potential future treatment for the chronic obstructive pulmonary disease [103]. Research carried out by Trotta *et al.* (2015) outlined the production of a formulation that met the criteria for inhalable therapies (*i.e.*, particle size, stability, sample recovery, adequate aerosol performance, uptake and transport). It was suggested that usage of the inhalation route would maximise tissue concentration of the drug in the lungs, resulting in a maximal therapeutic effect; however, bioavailability studies are yet to be performed to confirm this.

An additional investigated route of drug delivery RES is the transmucosal route [104, 105]. A recent study on RES lozenges examined their water solubility and bioavailability within 2 healthy male volunteers [105]. The study findings were indicative that RES-ribose lozenges possessed greater solubility compared to the alternative RES excipient formulations. This formulation achieved the maximum concentration (Cmax) of RES and rapid absorption into the bloodstream when compared to traditional gastrointestinal drug delivery. However, the research lacked comparisons with RES in its free form, thus acting only as proof of concept [105].

Alternative research has focused on enhancing the stability, solubility and permeability of RES *via* complexing nanosponges (NS) made from cyclodextrin [104]. These loaded sponges have exhibited desirable release and stability characteristics when tested *in vitro* against RES in its free form. Using rabbit mucosa, enhanced the accumulation of NS when compared to free RES. Moreover, further research using pig skin to determine permeation indicated NS to demonstrate ample permeation. Consequently, this formulation method has been suggested

as appropriate for buccal delivery or topical application of RES. These would, however, require further validation and testing in *in vivo* models prior to investigation in human subjects [104].

11. APPROACHES FOR OVERCOMING DELIVERY CHALLENGES

It has been suggested that the encapsulation of RES may be employed to improve its poor physiochemical properties (namely poor water solubility and stability); in turn, enhancing the compounds bioavailability
[106]. There are several prominent encapsulation technologies, which have been identified as suitable for overcoming the formulation difficulties associated with employing RES as an active pharmaceutical ingredient (API). Of particular interest are emulsion-based delivery systems, owing to their ability to encapsulate lipophilic therapeutic agents with the hydrophobic core of lipid droplets, offering protection from degradation during storage processes through to release following ingestion [107]. Factors for processing do, however, require consideration, including; the safe usage of safe excipients [108], insurance that the production process is not only robust and commercially viable but that the formulated product has favorable properties, is palatable, and has a sufficient shelflife. These may be considered generic challenges associated with any therapeutic compound. As compounds are constantly identified, the suitability of a drug delivery system is also of paramount importance and often required to be; site-specific, benign to the therapeutic compound, and biodegradable. Thus, irrespective of API efficacy, drug delivery systems are crucial in ensuring drug delivery to the desired target.

Nano-particulate-based drug delivery systems are an area of research, which has observed a surge in interest in preceding years. This is supported by 75% of scientific publications found in the discipline of nano-medicine relating to this area. Development of a drug delivery system, which possesses the transport ability with specificity to a given target, is an area of interest under constant investigation; as this is not only beneficial for new compounds but also may facilitate overcoming barriers with pre-existing compounds. As a parameter, solubility remains a well-documented barrier, poor solubility limits gastrointestinal absorption and thus subsequently bioavailability; with a number of strategies utilised in order to enhance solubility (*i.e.*, crystal alteration, size reduction, pH adjustment and spray drying).

Particulate lipid delivery systems are widely recognised and utilised as a remedy to issues surrounding compound solubility and membrane permeability, offering modi fied and target-specific release properties. This is in addition to superior stability, biodegradability and biocompatibility; versatility associated with excipients used and formulations; and a lower risk profile [109].

For Biopharmaceutics Classification System (BCS) class II drugs such as RES, an identified rate-limiting step is dissolution, influencing gastrointestinal absorption and subsequent bioavailability.

12. NANOTECHNOLOGY

In the preceding decades, a multitude of nanotechnologies have been developed in the field of biomedicine, each possessing unique characteristics and associate benefits, these include; nanoparticles (NPs), liposomes, micelles and the use of nano-coatings [110]. At present, the therapeutic applications of nanotechnology in the field of medicine are magnified around cancer diagnosis and therapy [111]. However, cardiovascular medicine is also a significant focus [112], as is antimicrobial resistance [113]. Table **1** summarises the nanotechnology applications in the treatment of cardiovascular diseases.

Recently, interest surrounding nanotechnology has grown exponentially, with researchers examining its use to increase the clinical efficacy of many naturally derived products, including RES. NPs exhibited potential in the enhancement of the bioavailability of various products by enhancing the stability of the therapeutic compounds interaction with biological systems [114, 115]. Additionally, in terms of physicochemical properties and targeted delivery, NPs enhance both the solubility and the transport across membranes [115, 116]. Common examples of this type of formulation include both Solid lipid nanoparticles (SLNs) [117] and nanostructured lipid carriers (NLCs), which have been observed to successfully improve RES oral bioavailability and preserve the compound from metabolic degradation; in addition to offering a controlled release profile [116]. This claim was supported by the formulation of SLNs in additional research [118].

Table 1. The Application of Nanotechnology in the Treatment of Cardiovascular Diseases [124].

Nanotechnology	Uses	References
Polymeric nanoparticles	Cardio-protectant	[125]
	Thrombosis	[126]
	Atherosclerosis	[127]
Nanocoating	Implants biocompatibility	[128]
Dendrimers	Cardio-protectant	[129]
	Thrombosis	[130]
	Atherosclerosis	[131]
	Vasodilation	[132]
Liposomes	Cardio-protectant	[133]
	Thrombosis	[134]
	Atherosclerosis	[135]
	Vasodilation	[136]

SLNs of RES have been utilised orally, augmenting drug plasma concentrations, and prolonging the duration of circulation. In the treatment of cardiovascular diseases, these characteristics are of great importance in order to achieve greater therapeutic outcomes. For example, the usage of SLNs to formulate Nimodipine (a calcium channel blocker used in the treatment of hypertension) to overcome its poor oral bioavailability (4-13%) was investigated in recent research [119]. Upon formulation, accelerated stability testing elicited no significant changes in size and polydispersity of the SLNs over a period of 3 months.

The usage of gold NPs conjugated with phytochemicals has been demonstrated to enhance anti-cancer properties in comparison to phytochemicals on their own [120]. *In vitro* trials, using RES gold NPs, were effective in blocking the advance in the MCF-7 cancer cell cycle when compared to RES alone and without cytotoxicity to healthy cells [121].

Additional research has focused on chitosan- and alginate-coated NPs; which have shown promise in controlling RES release and offering protection from UV degradation being a photosensitive compound [122]. The study findings suggest that the aforementioned NPs exhibit potential anticancer effects when administered topically or intravenously. However,

RES as a molecule and active agent still needs to be tested on animals in order to be able to extrapolate the data to humans [122].

Recent articles of the implementation of nanotechnology in the delivery of naturally derived compounds identified nanotechnology as being a potentially superior method in achieving chemoprevention/therapy, compared to current traditional methods [123]. However, a limited number of clinical trials are present which have tested such formulations. Moreover, nanotechnology is not free from pitfalls; it is associated with a number of disadvantages, including possible toxicity, potential crossing of the blood-brain- barrier, issues surrounding targeting and reduced half-life as a result of macrophage uptake in both spleen and liver [115]. However, despite the aforementioned drawbacks, the potential of nanotechnology in therapeutic treatments merits further investigation.

13. NOVEL FORMULATIONS

Grape-derived RES (RGC-RES) possessed a significantly higher bioavailability when compared to standard RES alone (Table **2**). Differences in structure (*i.e.*, the presence of glycosylation) provided enhanced stability as well as resistance to enzymatic degradation. In addition to the aforementioned properties, RCG-RES is associated with higher water solubility when compared to standard RES [137]. The enhanced properties in terms of solubility and oral bioavailability indicate RCG-RES to be a suitable substitute to RES in a clinical setting, though requiring further examination and clinical studies to investigate this further [137].

Table 2. Properties and Observations of RES Formulations in the Literature.

Formulation	Size (nm)	Observations	References
Microspheres	5µm	• Composed of Soybean PC, CHOL, coconut, and perfluorocarbons. • Improve resveratrol encapsulation and stability. • Protect the drug from light degradation	[138-140]

Cyclodextrins Nanosponges	5–10	• Increase resveratrol's aqueous solubility from 0.03 mg/mL to 1.1mg/mL • Improve resveratrol's cytotoxicity in HeLa, Hep3B and MCF-7 cell lines • Costly production process • Poor targeting in cancer therapy	[141-143]
Liposomes	100-120	• These formulations has increased aqueous solubility of resveratrol and Enhanced cytotoxicity in HeLa and HepG2 cell. • Improved efficacy and selectivity for HER2 over-expressing JIMT1 cells but not MCF-7 cells.	[144] [145]
Solid lipid Nanoparticles	150–586	• Simplicity of synthesis • low cost • intracellular delivery • improved solubility and stability of resveratrol *in vitro* • Poor drug loading presents a disadvantage for these formulations.	[116, 118, 146]
Polymeric nanoparticles	90–365	• Improved resveratrol absorption rate constant. • Enhanced cytotoxicity and cell uptake in DU-145 and LNCaP cell lines • Safe polymers • A disadvantage is its tedious and costly synthesis • Moderate drug loading	[147, 148]
Polymeric micelles	<100	• Good drug loading • Problematic to scale up synthesis techniques	[149]

14. NATURALLY OCCURRING RESVERATROL ANALOGUES

Many RES analogues hold superior effectiveness and pharmacokinetic profiles than RES. For example, RES trimethyl ether (trans-3,5,4'-trimethoxystilbene (RTE)) is a polyphenolic compound, which offers superior metabolic stability when compared to RES, with its clearance being significantly slower (8-9fold). Additionally, the plasma concentration of RTE's following intravenous or oral administration is shown to be greater than that of RES; this may

be attributed to the methoxylation of hydroxyl groups, enhancing the stability and resisting metabolism [150]. The oral absorption of RTE's majorly depends on oral solubility, which exhibited improvement when formulated as part of a drug delivery system, in some cases as β-cyclodextrin (β-CD). The more favourable pharmacokinetic profile of RTE offers further enhancement of its bioavailability through drug delivery system formulation; thus, further investigation of RTE as a beneficial agent is justified [150].

Pterostilbene (PTS) is an additional alternative RES analogue that has been demonstrated to be an appropriate candidate for further study. PTS possess a further desirable analogue pharmacokinetic profile, in comparison to RES. This is associated with its slower excretion, prolonged residence time and greater plasma bioavailability [151]. The above-mentioned property is attributed to PTS possessing a lower number of hydroxyl groups when compared to RES, reducing its susceptibility to conjugation and metabolism. The desirable pharmacokinetics of the analogue has been longestablished by research carried out by Choo *et al.* (2014), where PTS exhibited greater anti-inflammatory action in comparison to RES *in vitro*. Additionally, when examined, *in vivo* PTS was observed to accumulate substantially into main organs (*e.g.,* Brain, heart, liver, lungs and kidneys). PTS is thus a feasible alternative as a therapeutic agent, particularly as an anti-inflammatory agent [152].

A further alternative analogue of RES is Oxyresveratrol (trans-3,5,2',4'-tetrahydroxystilbene, (OXY)); this possesses an extra hydroxyl group on the aromatic ring, resulting in a greater water solubility when compared to standard RES. Research conducted by Chen *et al.,* (2016) investigated the OXY and RES pharmacokinetic profile in rats. The mean transfer time of OXY was equivalent to that RES, however, OXY was also found to possess greater oral bioavailability and was absorbed significantly faster with slower clearance than RES, suggesting a further investigation into the effectiveness of OXY as a curative agent [153].

CONCLUSIONS

The growing incidence of CVD states globally is a significant cause of concern. Novel agents such as RES are under significant demand to offer efficacy, in addition, to ease in drug delivery. Whilst, this review has demonstrated that there is clear evidence that RES offers a significant degree of cardio-protection and is useful in the treatment of cardiovascular

diseases. It cannot be overlooked that the drug presents significant challenges in terms of bioavailability and water solubility. Though partially remedied by the discovery/development of analogues with various characteristics and also the formulation of the drug in various nanoparticle formulations to facilitate delivery and targeting; there is a definitive lack of clinical evidence in the form of clinical trials, animal and human, to further validate this evidence. If RES is to hold a place in the prevention, treatment and adjuvant therapy of CVD states and battle against cancer, this must be addressed through early detection, diagnosis and efficient targeting process.

LIST OF ABBREVIATION

WHO = World Health Organization

CVDs = Cardiovascular Diseases

NCDs = Non-Communicable Diseases

LMICs = Low and Middle-Income Countries

HF = Hart Failure

RES = Resveratrol

UV = Ultraviolet

STS = Stilbene Synthase

CoA = Coumaroyl-Coenzyme

GLU-RES = Resveratrol Glucuronide

SUL-RES = Resveratrol Sulphated

AMPK = AMP-Activated Protein Kinase

SIRT-1 = Sirtuin 1

Nrf2 = Nuclear Factor (Erythroid-Derived 2) Like 2

NF-B = Nuclear Factor-Kappa B

LDL = Low-Density Lipoproteins

HMG-CoA Reductase = 3-Hydroxy-3-Methyl-Glutaryl-CoAreductase

LDL-R = Low-Density Lipoproteins Receptors

NO = Nitric Oxide

Ang II = Angiotensin II

Ca2+ = Calcium

ACE = Angiotensin-Converting-Enzyme Inhibitors

CAD = Coronary Artery Disease

MI = Myocardial Infarction

SGLT = Sodium-Glucose Cotransporter

VCAM-1 = Vascular Cell Adhesion Molecule-1

IL = Interleukin

TNF = Tumor Necrosis Factor

CRP = C-Reactive Protein

ICAM-1 = Intercellular Adhesion Molecule 1

PGC-1a = Peroxisome-Proliferator-Activated Receptor Co-Activator

DNA = Deoxyribonucleic Acid

API = Active Pharmaceutical Ingredient

BCS = Biopharmaceutics Classification System

SLNs = Solid Lipid Nanoparticles

NLCs = Nanostructured Lipid Carriers

MCF-7 = Breast Cancer Cell Lines

PC = Phosphatidylcholine

CHOL = Cholesterol

RTE = Resveratrol Trimethyl Ether

PTS = Pterostilbene

OXY = Oxyresveratrol

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