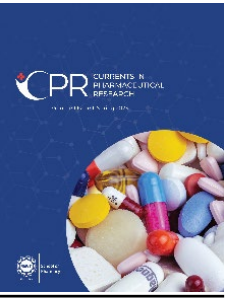
Currents in Pharmaceutical Research (CPR)
Volume 2 Issue 2, Fall 2024
ISSN$_{(P)}$: 3007-3235, ISSN$_{(E)}$: 3007-3243
Homepage: https://journals.umt.edu.pk/index.php/cpr

Article QR



Title:	**An Overview of Anticancer, Anti-inflammatory, Antioxidant, Antimicrobial, Cardioprotective, and Neuroprotective Effects of Rutin**
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DOI:	https://doi.org/10.32350/cpr.22.06
History:	Received: July 05, 2024, Revised: August 15, 2024, Accepted: October 03, 2024, Published: November 15, 2024
Citation:	Azeem M, Niazi S, Fayyaz F, et al. An overview of anticancer, anti-inflammatory, antioxidant, antimicrobial, cardioprotective, and neuroprotective effects of rutin. *Curr Pharma Res*. 2024;2(2):119–155. https://doi.org/10.32350/cpr.22.06
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Conflict of Interest:	Author(s) declared no conflict of interest.

A publication of
The School of Pharmacy
University of Management and Technology, Lahore, Pakistan

An Overview of Anticancer, Anti-inflammatory, Antioxidant, Antimicrobial, Cardioprotective, and Neuroprotective Effects of Rutin

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ABSTRACT

The current review focuses on the biological role and potential benefits of rutin, a natural flavonoid, in medical and pharmaceutical fields, especially for the treatment of different disorders in human beings. Rutin is present in various fruits and vegetables, such as tobacco, forsythia, buckwheat viola, apricots, cherries, grapefruit, plum, oranges, Japanese pagoda tree, eucalyptus macrorhyncha, St. John's Wort, ginkgo, apples, and hydrangea as vitamin P. Its absorption is constrained by the gut flora, thus it is less bioavailable. However, in consolidation with other drugs, rutin shows enhanced properties such as anti-inflammatory, antioxidant, antimicrobial, cardioprotective, neuroprotective, and anticancer properties. Literature provides little data about its activity in cosmetics as antiaging, as well as its use in ulcerative colitis, autism, animal food, and pregnancy. Although several studies have revealed its activity against SARS-CoV-2. For this study, literature search was performed using various tools including books, Google Scholar, PubMed, and SpringerLink. A total of 370 research papers were studied. It was observed that 35% were about anticancer, 20% were about antioxidant, 15% were about anti-inflammatory activity of rutin, while 10% each were about antihypertensive, antimicrobial, and neuroprotective effects of rutin.

Keywords: anti-inflammatory, antioxidant, antimicrobial, anticancer, cardioprotective, neuroprotective, vitamin P

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1. INTRODUCTION

Rutin, a plant pigment also known as Rutinoside, is a polyphenolic compound with low molecular weight. It is obtained from plants such as tobacco, forsythia, buckwheat viola, apricots, cherries, grapefruit, plum, oranges, Japanese pagoda tree, eucalyptus macrorhyncha, St. John's Wort, ginkgo, apples, hydrangea, and some other vegetables and fruits [1, 2]. It is found in large quantities in the family Rutaceae, especially in *Ruta graveolens* (rue). About 3-4% of the rutin yield is obtained from plants such as sophora, tobacco, and *Eucalyptus* spp. (buckwheat) and used for commercial purposes. The flowers of *Sambucus nigra* containing p-coumaric acid, kaempferol, and rutin have been used in domestic and veterinary medicine, especially in ointments. Albert Szent Györgyi, a Hungarian scientist, discovered rutin (vitamin P) and vitamin C. He also claimed to successfully treat bleeding disorders with citrins (vitamin P). However, further research on flavonoid preparation of hesperidin and rutin did not prove this claim [3].

Rutin has a varied solubility profile depending upon the pH of the medium. It shows very limited solubility in water, but its solubility can be enhanced as pH is changed to alkaline. As the pH changes, it rapidly hydrolyses, yielding quercetin, rhamnose, and glucose. Rutin has a crystalline shape, soluble in alkali [3]. When exposed to light, rutin converts into a dark powder form and also shows hygroscopic properties. Recrystallized rutin from the water gives pale yellow needle-shaped crystals [4]. Various methods including maceration, percolation, extraction under reflux, and soxhlet apparatus are used for the extraction of rutin. Its extraction shows an increased concentration of other naturally occurring compounds in plants by a transformation which is dependent on the pH of the extractant, concentration of alcohol, heating time, and matrix components of plants [5].

Rutin is a naturally occurring citrus flavonol glycoside derivative. Flavonoids are compounds with a phenyl benzopyrone ring. When flavonoids combine with one or more sugar molecules, they produce flavonoid glycoside. Whereas, their uncombined form is called aglycone. Flavones either occur as C and O glycosides or in a free state. They impart colour to fruits and flowers and act as stress modifiers, powerful antioxidants, antiviral, anticancer, anti-allergen, diuretics, anti-spasmodic, hypolipidemic, anti-bacterial, anti-fungal, and vasoprotective agents in

human beings [1, 6]. They are also used as colorants, stabilizers, and preservatives in the food industry. In addition, they have applications in herbal medicines, multivitamins, cosmetics, and animal foods [1].

The best known flavonoid constituents are rutin, citrus bioflavonoids, and quercetin. Rutin and hesperidin are also considered as permeability factors. Vitamin P is used in the treatment of increased capillary fragility and capillary bleeding. Citrus bioflavonoids are also used in treating common cold and dietary supplements [7]. Rutin's bioavailability is constrained because of its low aqueous solubility, stability, and membrane permeability [4]. When taken orally, gut microbiota metabolizes it and absorption takes place through the intestine. Rutin is bioavailable as conjugated metabolites. However, it is less absorbed because of the metabolism in the gut flora [8].

Based on their structure, flavonoids are classified into six subgroups: flavonols, flavanones, flavones, isoflavones, flavan-3-ols, and anthocyanidins. Some flavonoids, originating from flavones, are classified as flavonoid glycols, flavanones, isoflavones, xanthones flavonoids, and flavonols [6]. Figure 1 shows the chemical reaction of quercitin and rutinose, resulting in rutin formation.

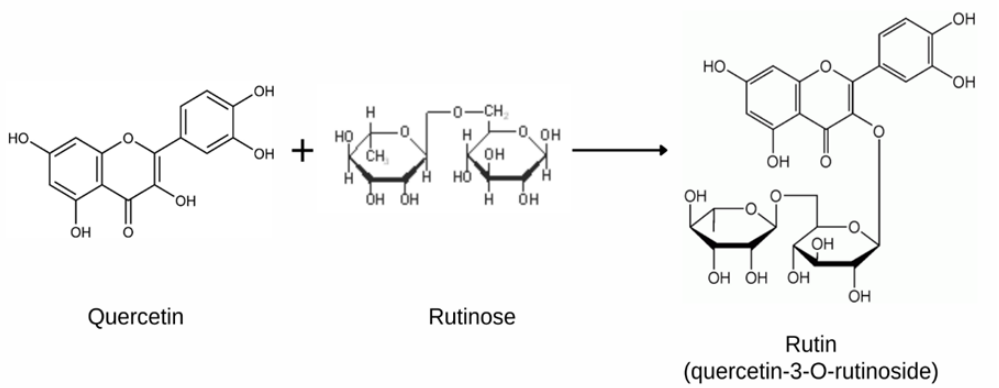

Figure 1. Hydrolytic Reaction of Quercetin and Rutinose Resulting in Rutin Formation (Quercetin-3-O-Rutinoside)

Rutin shows minor side effects as an anticancer agent, as compared to synthetic chemotherapeutic drugs which show many side effects. Moreover, it regulates various cellular signaling pathways (Apoptosis, p53-independent pathway, P13k / Akt, JAk / STAT, Wnt/B-catenin, MAPK, NF-kB and p53 etc.) related to carcinogenicity to mediate the influence of

anticancer agents [9]. In human body, Cytochrome P-450 enzymes are essential in determining the administered drug's bioavailability. Enzymatical activity is affected by phytochemicals. Rutin, in combination with anti-diabetic drugs, decreases drug bioavailability and increases drug metabolism by inducing CYP3A4 enzyme activity. In this way, it decreases the anti-diabetic effect of the drug [10]. It also showed cardioprotective benefits, both in *in vitro* and *in vivo* studies, by cardiac remodeling of various processes, such as autophagy, protein synthesis, hypertrophic signaling, and oxidative stress. Therefore, it has the potential to be formulated for adjunctive clinical therapy in heart failure and hypertrophic cardiomyopathy [11]. Furthermore, its combination with other drugs showed a significant increase in the inhibition of cell proliferation and apoptosis, synergistic induction, and cancer cell cycle, including the inhibition of breast, colon, lung, and prostate cancer cells and other tumors. The combination caused less resistance to the therapeutic drug and has few side effects [12].

Rutin is highly effective in treating a wide range of neurodegenerative disorders, particularly Alzheimer's disease, Parkinson's disease, prion disease, and Huntington's disease. These disorders are caused by the loss of neurons, inflammation, loss of mitochondria, oxidative stress, and apoptosis [13, 14]. Moreover, rutin has cytoprotective properties due to the presence of polyphenolic structures. It protects the skin against UV-induced harm and also provides protection against apoptosis [15]. A study showed the cytoprotective activity of rutin and ascorbic acid. They are mostly used in combination and are intended for oral use. They are used for the protection of skin cells from the harmful effects of sun's UV radiations. Rutin and ascorbic acid complex prevent the modification of a protein by lipid peroxidation [16]. It also showed its effect on DNA damage by oxidation. Furthermore, it is a cytoprotective agent against chemical induced toxicity, inflammation, and oxidative stress. Moreover, it also demonstrates neuroprotective effects by serum glucose impoverishment [17].

Polycyclic flavonoids show anti-inflammatory and anti-allergic activities useful for treating asthma. The inhibition of neutrophil elastase, mast cells, and cytokines are the various mechanisms proposed as anti-inflammatory and anti-allergic agents [18]. In a recent study, a herbal drug containing rutin was investigated for its antiproliferative effects in mast

cells by reducing its proliferation without being exposed to cytotoxicity. Further, rutin treatment also reduced the levels of cytokines [19].

In a recent study, the antiplatelet effect of various flavonoids including quercetin, rutin, diosmetin, and diosmin was observed. Rutin plays a vital role in vascular disorders as an antiplatelet agent. Although the exact mechanism remains partially unknown, flavonoids mainly prevent capillary penetrability and improve blood flow. These flavonoids were also investigated for platelet activation. The study demonstrated that antiplatelet activity was due to the blockage of glycoprotein IIb/IIIa receptors, as well as due to the inhibition of platelets along with a reduction in calcium ionophore activity [20]. Another study was performed on the antiplatelet effect of flavonoids as inhibitors of cyclooxygenase-1. The antiplatelet activity of rutin is mediated by prostaglandins that affect arachidonic acid flood and inhibit the cyclooxygenase enzyme [21].

A study was performed on rat models to evaluate the potency of rutin in the prevention of surgically caused endometriosis, which can be treated by apoptosis and antioxidant mechanisms. Rutin showed antioxidant activity that induces apoptosis by formulating Caspase, Bax, and Bcl-2 and also causes a rise in antioxidant concentration [22]. Alpha glucosyl derivative of rutin increases water solubility and is used in cosmetics and food [23]. Frozen dried nanocrystals of rutin were developed in order to increase their solubility. These were further re-dispersed in carbopol gel to enhance transdermal delivery. They have an excellent anti-photoaging effect [24].

Rutin shows significant therapeutic activity in inflammatory bowel disease. It is delivered as a prodrug that releases active quercetin at the site of inflammation. The mechanism of action may include the suppression of pro-inflammatory mediators, while the expression of inflammatory proteins has been investigated as well. Natural therapy is effective because the current drug treatment has inherent problems [25].

Aging is a complex process which is due to gradual changes in skin and extrinsic factors that cause structural and functional changes. The external factors include free radicals, UV exposures, and pollution. Rutin and caffeic acid show antiaging effects because of their free radical trapping effects [26]. Malignant melanoma is a type of skin cancer that is fatal and has few treatment options in advanced stages. Herbal constituents with antitumor effects are considered very important in melanoma treatment as

chemotherapeutic agents. Rutin, a naturally occurring agent has antioxidant, antimicrobial, anti-inflammatory, UV filtering, and sun protecting factor (SPF) enhancing effects that are advantageous to the skin [27]. SPF value is increased by rutin which makes it valuable to be used in sunscreen. *In vitro* studies showed that rutin increases the SPF value of sunscreens, providing dual action of antioxidant and sun protection [28].

Rutin has the ability to play a role by itself, but in combination with other drugs its pharmacological effects are enhanced. In order to achieve synergistic effects, various combinations of rutin are available. There is still a need to establish the pharmacokinetic and pharmacodynamic profile of various combinations of rutin in order to determine its half-life, Cmax, Tmax, and other properties. Further, there is a need to develop different dosage types of rutin. More work is also needed to evaluate the steady state concentration and toxicity of rutin. Most of the previous studies focused on its anticancer, anti-inflammatory, anti-oxidant, antimicrobial, neuroprotective, and cardioprotective effects, but it also has antiallergic, cosmetic, and other uses that need to be studied in detail in order to understand its multipurpose drug properties. Only a few studies have illustrated the role of rutin when incorporated in the pharmaceutical drug delivery system. Most of these studies focused on the extraction and phytochemical analysis of the plant. On the contrary, this review article focuses on the potential benefits of rutin in pharmaceutical and nutritional sciences. Further studies should be performed to assess its solubility issues and bioavailability profile.

1.1. Identification Tests for Rutin

Table 1 mentions the general chemical tests performed for the detection of flavonoid glycoside. While, Figure 2 depicts a schematic diagram illustrating the search strategy for the literature survey regarding the potential benefits of rutin.

Table 1. Identification Tests Performed for the Detection of Flavonoid Glycosides Including Rutin [4, 29].

Identification Tests	Method	Indications
Ammonia test	Dip filter paper in an alcohol solution and then	Yellow spots

Identification Tests	Method	Indications
	expose it to ammonia vapours.	
Shinoda Test	Add diluted HCl to the drug magnesium (turnings) alcoholic extract.	Appearance of red colour
Vanillin HCl Test	Add vanillin HCl to the drug alcoholic solution.	Appearance of pink colour
Bornträger Test	Step 1: Dissolve the extract in water and then filtrate it. Treat filtrate with NaOH. Step 2: Add a few drops of $H_2SO_4$to NaOH treated extract.	Appearance of yellow colour in alkaline phase (NaOH) and it disappears in the acidic phase (H_2SO_4).
Neutral Lead Acetate Test	It contains 10% lead acetate + ethanol + extract.	Orange precipitates
Lieberman Burchard Reagent	Add acetic anhydride + H_2SO_4 in a dry test tube containing chloroform. Mix well and then allow it to stand for a few minutes.	Appearance of pink colour

1.2. Literature Survey Method

An extensive data review was carried out between May 2022 and August 2022 of studies available on PubMed, ScienceDirect, Springer books, Wiley Online Library, and Google Scholar. Search keywords included rutin, neuroprotective, anticancer, cardioprotective, antioxidant, anti-inflammatory, and antimicrobial effects of rutin, both alone and in combination. A total of 370 articles were assessed. After detailed screening,

63 articles were selected for review and the remaining 308 were excluded. The included articles were relevant to pharmaceutical dosage form, along with a treatment strategy of rutin, both alone or in combination with other drugs. The excluded articles were more focused on phytochemical analysis, extraction, and herbal treatment of rutin.

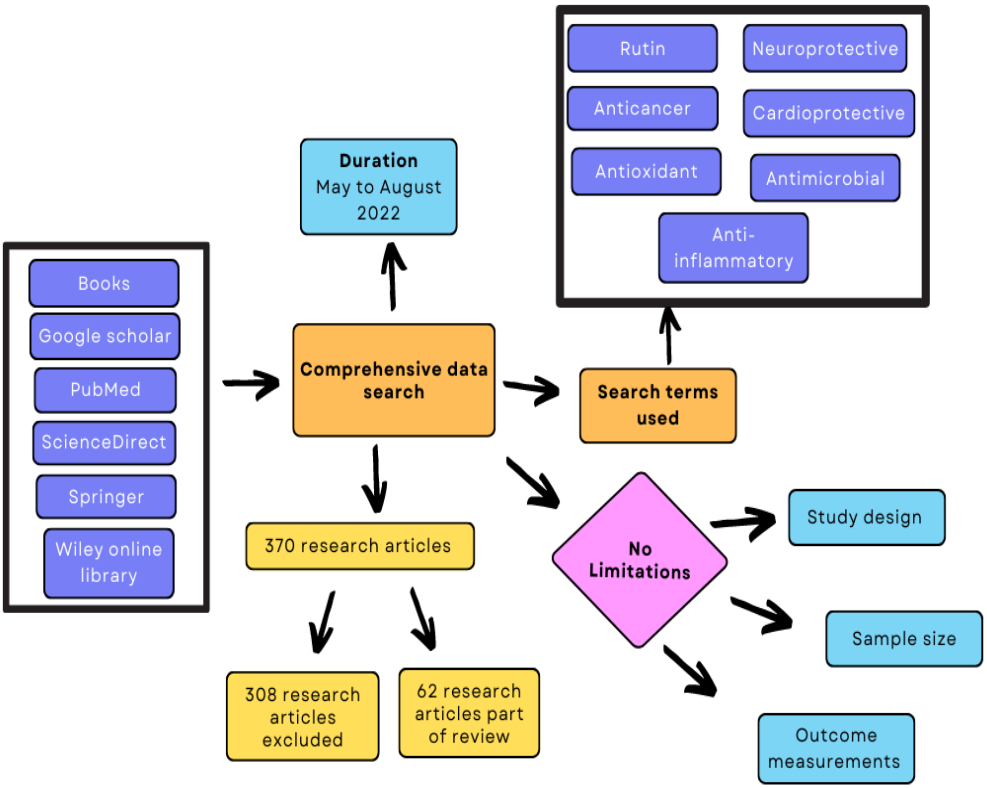

Figure 2. Search Strategy Flow Chart

1.3. Rutin Sources

Rutin is naturally found in a variety of vegetables, fruits, and non-edible plants. About 10.53% was found in the non-edible plant *Rhus cotinus* [30]. Moreover, 8.4% of rutin was found in *Sophora secundiflora* seeds and 5.2% in *Mangifera indica* leaves [31]. Furthermore, 2.76%, 1.8%, and 0.28% rutin was found in leaves, flowering buds, and fruits of *Capparis spinosa L.,* respectively [32]. Table 2 shows the amount of rutin found in different sources.

Table 2. Fruits and vegetables Having Different Percentage of Rutin [30].

Sources	Percentage of rutin (%)
Apple	0.17
Sweet cherry	0.18
Morello cherry	0.18
White cherry	0.15
Aronia	0.34
Red pepper	0.17
Red hot chillies pepper	0.22

2. PROPERTIES OF RUTIN

2.1. Anticancer Property

Prasad R and Prasad S confirmed anti-tumour activity and protection against hemotoxicity, when rutin and cisplatin are used together. Rutin (CAS No. 153-18-4, rutin hydrate) with more than 94% purity was purchased. Afterwards, its freshly prepared solution in DMSO at the concentration of 1mg/20ml was diluted in PBS (phosphate buffer saline) to get the desired concentration. DMSO percentage in the desired concentration was kept less than 0.5%. The authors performed *in vivo* studies on an inbred Swiss albino mice colony and were intra-peritoneally transplanted with Ascites Dalton's lymphoma (DL cell - 1×10^7 i.p injection). Then, they were treated alone and in combination with rutin and cisplatin. The results revealed that the combination treatment showed rutin protective effects against cisplatin-induced hemotoxicity caused by a decrease in the level of hemoglobin (Hb), former leucocyte count, and erythrocytes. Also, there was observed 57% increase in lifespan and host survivability by causing a further decrease in the reduced level of glutathione in tumour cells [33].

Caparica R and coauthors confirmed the anticancer activity of rutin-ion liquids on renal cancer cells. *In vitro* studies were performed on Vero-E6 (normal renal cells) and 786-O (renal cancer cells) of human beings, obtained from American Type Culture Collection (ATCC). The results revealed that rutin markedly increased the Sub G-1 population of 786-O cells, causing a decrease in its viability. Moreover, Vero cells didn't show any cytotoxic effect at 50µM.

The solubility of rutin in water is very low at 0.2 mg/ml, which limits its applicability by making its incorporation difficult in delivery systems. Rutin, in combination with ionic liquids (ILs) and 2-choline amino acid, showed improved solubility, resulting in an increased anticancer effect. This shows that poorly soluble rutin, when added to the IL-nanoparticle hybrid system, performs controlled drug delivery which enhances its anticancer properties [34].

Dixit S performed *in vitro* studies on Swiss albino mice to evaluate the topical antitumor effect of rutin obtained from the methonolic extract of *Triticum aestivum* straw. Albino mice were treated topically with 7, 12-dimethyl benzanthracene (DMBA) and croton oil to induce skin cancer. To prevent skin carcinogenesis, rutin was administered orally for 16 weeks (3 times a week) at a dose concentration of 40 mg/kg and 200 mg/kg body weight. The results revealed greater reduction in the cumulative number of papillomas, tumour size, reduction in serum glutamate oxalate transaminase levels, bilirubin, alkaline phosphatase, reduction in serum glutamate pyruvate transaminase, and lipid peroxidase enzymes. It significantly increased enzyme level involved in catalase superoxide dismutase and oxidative stress glutathione [35].

Jayameena P et al confirmed the anti-cancer activity of rutin against human colon cancer cells HCT116. Rutin was standardized by MTT assay against HCT116 and HacaT cells. The results showed a reduction in migration in G0-G1 phase (arrest cell cycle) and improvement in β-actin and caspase-3 expression of HCT116 cells. This study strongly claimed colon cancer cells inhibition by rutin and postulated its use as a beneficial alternative to chemical therapeutic drugs [36].

Bohlouli S et al examined the preparation, characterization, and evaluation of rutin nanocrystals against squamous cell carcinoma cell line of head and neck. The ultrasonication method was used to prepare rutin nanocrystals and conventional techniques were used to determine their physicochemical characteristics. The results concluded that rutin nanocrystals showed 100 times more cytotoxic effect than free rutin on the cells of HN5, leading to an increased Bax/Bcl ratio due to mitochondria-dependent apoptotic pathway within the median inhibitory concentration (IC50) of 30.5µm in 24hrs incubation time. Besides, no marked cytotoxic effect was observed on HGF1-PI1 (normal oral cells) at 24hrs and 48hrs incubation, when treated with rutin nanocrystals [37].

Chang C et al, performed an *in vitro* research that utilized ionic gelation process to make nano-conjugates of rutin-chitosan for anticancer and apoptotic induction in TNBC cells. These non-conjugates were characterized by XRD, DLS, SEM, FTIR, UV–Vis Spectra, and DAPI fluorescence micrographic analysis and zeta potential. The results showed significant DNA synthetic phase cell cycle arrest and apoptotic cell death of nano-conjugates at the inhibitory concentration of 12.5µg/ml [38].

2.2. Anti-inflammatory Property

Modi FD et al reported the anti-inflammatory effect of rutin *in vivo* after its intramuscular administration (100mg/kg) to albino Wister rats weighing 300-400g in carrageenan-induced oedema assay. HPLC was used to determine the Cp of rutin and its pharmacokinetic parameters were studied (Cmax, Tmax, Cl, $H\frac{1}{2}$, Vd, MRT). The results stated that rutin reduced 29.94% edema and inflammation in animals after 6hrs of drug administration. The observed plasma concentration was 0.21 ± 0.02µg/ml, calculated during 24hr time interval [39].

Song H et al reported rutin's anti-inflammatory and antioxidative mechanisms. Moreover, p38 mitogen (p38 MAPK) inhibition and activated protein kinase pathway play their vital role in performing neuroprotective activity in the treatment of spinal cord injury, as established by Allens's method in Sprague Dawley rats. For 3 days, the rats were injected with 30mg/kg of rutin intraperitoneally, increasing locomotive function scores of Beattie, Basso, and Bresnahan, while markers related to oxidative stress, superoxide-dismutase (SODs), glutathione peroxidase (GPx) level, p38 MAPK protein expression, interleukin-1β (IL-1β and 6 levels), water contents, tumour necrosis factor-α (TNF-α), caspase-3 and 9 activities decreased significantly at T_{8-9} spinal cord [40].

El Gizawy HA et al reported different experiments performed both *in vitro* and *in vivo* along with molecular docking and dynamics on *Pimenta dioica*, ethyl acetate extract, and bioactive constituents including rutin, gallic acid, chlorogenic acid, and ferulic acid, exhibiting their anti-inflammatory and anti-SARS-CoV-2 activities. Anti-inflammatory and antiviral agents are essential in managing COVID-19 patients. This is because in viral infections, cytokines in immunological responses often lead to acute respiratory distress. *Pimenta dioica* bioactive compounds were isolated from leaves, identified using spectroscopy techniques, and analysed

for the presence of CC_{50} (cytotoxic activity for half maximal) and IC_{50}(SARS-CoV-2 inhibitory concentration). After treating the lung toxicity among rats induced by mercuric chloride with *P. dioica* extract and its active constituents, the results showed promising anti-inflammatory and anti-SARS-CoV-2 activity of rutin and ferulic acid having IC_{50} values of 31µg/ml (rutin), 108µ/ml (gallic acid), and 360µg/ml (chlorogenic acid), respectively [41].

Chunlian Tian C et al performed *in vitro* studies determining rutin's anti-inflammatory, antioxidant, and free radicals action on lipopolysaccharide-induced RAW-264 (murine macrophages) to examine the dose-effect relationship. The determination of cell morphology contents (NO, TNF-α, IL-1β and IL-6) and phagocytic activity was performed to assess rutin's anti-inflammatory activity. The results showed that rutin exhibited stronger anti-inflammatory activity at 50µm and 100µm and antioxidant activity than butylated hydroxytoluene (BHT) and VC drugs [42].

Nikfarjam BA et al investigated rutins' anti-inflammatory activity on neutrophil-mediated and auto-immune diseases, utilizing peripheral blood neutrophils of humans isolated using the density gradient centrifugation (Ficoll-Hypaque) technique and cultured in Roswell Park Memorial Institute medium. The culture medium was pre-incubated for 45mins. ELISA was used to analyse TNF-α, NO, and MPO (myeloperoxidase) production viability with MTT (Mean Transient Time) assay. Further, 1µm-100µm rutin in various concentrations were used to treat neutrophils. Later on, MTT substrate was added and incubated for 24hrs at 37°C, resulting in increased anti-inflammatory property of rutin by inhibiting TNF-α, NO, and MPO activity and significantly decreasing their production in activated human neutrophil cells [43].

Cosco D et al performed *in vitro* studies using the technique of spray drying for analyzing anti-inflammatory activity of rutin. The evaluation of rutin and NCTC-2544 and C-28 cells was done by treating them with lipopolysaccharide and their IL-1β and IL-6 levels were evaluated. The results showed that the treated cells cytosol compartment was localized with drugs with an increase in anti-inflammatory activity of chitosan loaded with rutin, as compared to free rutin [44].

Zapata-Morales JR et al, performed activities to assess the synergistic profile of rutin with NSAIDs and with paracetamol tested in RAW 264.7 macrophages. The results on isobolograms showed that *in vitro* rutin-NSAIDs and rutin-paracetamol combinations exhibited synergistic effects. The interaction index of rutin-diclofenac showed the value 0.17, while in the *in vivo* assay, rutin-diclofenac and rutin-ketorolac showed the values 0.195 and 0.408, which exhibited synergistic effects, while rutin-paracetamol showed additive effects [45].

2.3. Antioxidant Property

Enogieru AB et al reviewed rutin's mode of action as a neuroprotective agent in many experimental models of neurodegenerative diseases. Rutin acts as a neuroprotective or antioxidant by causing reduction in pro-inflammatory cytokines, increasing the activity of oxidative enzymes inducing mitosis, reducing mRNA expression, elevating anti-apoptotic and ion transport genes, and re-imposing mitochondrial activity. Intravenous delivery of congored-rutin magnetic NPs resulted in memory recovery with less neurologic loss in the brains of transgenic mice APPswe/PS1dE9 [14].

Further studies were conducted by Gegotek A et al on the anti-apoptotic and antioxidant activity of rutin and ascorbic acid in combination with keratinocytes and fibroblasts of human beings exposed to ultraviolet radiation (A and B). Skin cells (fibroblasts and keratinocytes) were subjected to ultraviolet radiation (A and B). The study aimed to assess the synergistic profiles of ascorbic acid and rutin, as well as to measure their combined antioxidant profile along with their effect on cellular antioxidant level, oxidation of lipid and proteins, and transmembrane transport and expression of pro-inflammatory and anti-apoptotic protein. The results revealed enhanced antioxidant effect of rutin in combination with ascorbic acid. Rutin showed great potency against UV-induced ROS when given in combination with ascorbic acid. The collaborative effect of rutin and ascorbic acid suggested the potential antioxidant and anti-apoptotic activity against UV-induced skin damage [46].

Nandana CN et al performed a study on the synthesis of chitosan/silver nanostructures of rutin. The bio-inspired method was used to develop chitosan/silver nanocomposite. The objective was to develop nanocomposites for their antibacterial, antioxidant, and photocatalytic effects. The formation of chitosan/silver nanostructures was confirmed by

the development of brown colour and UV-visible absorption peak at 415nm. XRD analysis revealed crystalline peaks of synthesized nanocomposites. Spherical-shaped nanostructures with an average size of 23-78nm were observed by using field emission-scanning electron microscope. Further, functional groups of chitosan and rutin were observed in Fourier transformed infrared spectroscopy. Spectrophotometrically, the *in vitro* antioxidant activity of nanocomposites was observed by using the DPPH method. The results revealed that the antioxidant profile was enhanced by enhancing the amount of chitosan/silver nanostructures. The synthesized nanostructures had a high disc diffraction antibacterial effect against *Bacillus subtilis* and *Escherichia coli.* Photocatalytic activity of nanocomposites was assessed by using sunlight to remove methylene blue from the aqueous solution. The results indicated that the degradation of methylene blue dye was 88% under sunlight irradiation for 220 mins. Thus, the synthesized chitosan/silver nanostructures using rutin can be used for multiple functions [47].

Wani TA et al performed a study to assess the antioxidant profile of flavonoids, rutin, and quercetin by binding neratinib to human serum albumin *in vitro*. The research aimed to investigate the binding effect of neratinib with human serum albumin in the presence of antioxidant flavonoids, namely rutin and quercetin. In order to investigate this *in vitro* interaction, molecular docking and by using different spectroscopic analysis were performed. The interaction between human serum albumin and neratinib was observed by using static fluorescence quenching mechanisms. The results indicated that rutin and quercetin caused changes in the binding constant between human serum albumin and neratinib and thus affected their binding. These flavonoids bind competitively with human serum albumin and hence displace neratinib [48].

Ravi GS et al performed a study to formulate a nano-lipid formulation of rutin. The study aimed to enhance the solubility profile and plasma concentration of rutin and to investigate the effect of the nano complex of rutin on hepatoprotective and antioxidant activity. For this purpose, evaporation, precipitation, and lyophilization were used to formulate the nano complex and then their comparitive analysis was performed. The *in vivo* study was performed on rats to investigate hepatoprotective and antioxidant activity and rutin's bioavailability. After optimization, standard solvent DMSO, co-solvent (butanol), and rutin at EPC ratios of 1:1, 1:2, and

1:3 were chosen for lyophilization. Nano compounds were characterized for size, charge, surface behavior, complexation, thermal analysis, drug content, solubility profile, and *in vitro* and *in vivo* analyses for drug release, as well as antioxidant and hepatoprotective effects. By using the lyophilization method, nano complexes of rutin were formulated in the nano-size range. The results indicated enhanced solubility and drug release with a diffusion-controlled release profile confirmed by kinetic models. Nano complexes showed enhanced antioxidant activity during *in vitro* analysis and also better stability in different pH media *in vitro*. On the other hand, *in vivo* analysis revealed enhanced hepatoprotective activity along with increased bioavailability of nano complexes in comparison to pure rutin (at the same dose). Group II animals treated with carbon tetrachloride failed to maintain the normal levels of serum hepatic enzymes along with liver antioxidant enzymes. Thus, the formulated nano rutin complex showed enhanced bioavailability, solubility, and antioxidant profile [49].

Lins TLBG et al performed an experiment to analyze the antioxidant activity of rutin and the regulation of P TEN and FOXO3a phosphorylation in the prevention of ovarian damage induced by cisplatinin mice. One group of mice was given saline solution 0.15M and cisplatin 5mg/kg intravenous, while the positive control group was treated with N-acetylcysteine 150mg/kg orally or with rutin 10, 30 or 50mg/kg orally before cisplatin treatment, once daily for 3 days. Histological, immunohistochemical, and fluorescence examination of the ovaries was performed. Further, the molecular mechanism through which rutin prevents ovarian damage induced by cisplatin was analyzed by calculating the expression of FOXO3a and PTEN phosphorylation. The results indicated that the mice pretreated via acetylcysteine or 10mg/kg rutin prior to cisplatin showed normal cell proliferation and follicle percentage, decreased programmed cell death, and increased ROS with the enhancement of mitochondria and glutathione, as compared to cisplatin. In follicles, cisplatin enhanced the p-PTEN and reduced the p-FOXO3a expression, whereas 10mg/kg rutin prevented this. Hence, it was concluded that 10mg/kg rutin treatment prevents ovarian damage induced by cisplatin via antioxidant effect and PTEN/FOXO3a pathways [50]. The summary of anticancer, anti-inflammatory, antioxidant, cardioprotective, neuroprotective, and antimicrobial properties of rutin is shown in Table 3.

Table 3. Rutin as Anti-Oxidant, Cardioprotective, Anti-Cancer, Anti-Inflammatory, Neuroprotective, and Antimicrobial Agent

Uses of Rutin	Therapeutic Agent	Cell Lines and Assays Used	Outcomes	Reference
Anti-cancer	Rutin and cisplatin	Dalton's lymphoma	Enhanced anti-cancer activity due to reduced glutathione level and cisplatin induced hemotoxicity.	[33]
	Rutin-ion liquids	Renal cancer	Enhanced anti-cancer activity	[34]
		Skin cancer cells	Reduction in papillomas cumulative number, tumor size, serum glutamate, oxalate transaminase, bilirubin, alkaline phosphatase, pyruvate transaminase, and lipid peroxidase enzyme. Increased catalase peroxidase dismutase.	[35]
Anti-inflammatory	Rutin	Colon cancer	Colon cancer cells inhibition.	[36]
	Rutin nanocrystals	Squamous carcinoma	Shows 100 times more cytotoxic effect than free rutin and increased ration of Bax/Bcl.	[37]

Uses of Rutin	Therapeutic Agent	Cell Lines and Assays Used	Outcomes	Reference
	Rutin-chitosan nanoconjugates	Triple-negative breast cancer	Significant DNA synthetic phase cell cycle arrest and apoptotic cell death.	[38]
	Rutin	Edema	Inhibition of inflammation and reduction in oedema volume.	[39]
	Rutin	Spinal cord injury	Increased anti-inflammatory and anti-oxidant activity. Activation of protein kinase pathway enhancing neuroprotective effect.	[40]
	Rutin and ferulic acid	Lung toxicity	Increased anti-inflammatory and anti-SARS-CoV-2 activity.	[41]
	Rutin	Murine macrophages and free radicals or ions	Stronger anti-inflammatory activity than BHT and VC drugs.	[42]
	Rutin	MTT assay, neutrophil mediated and auto-immune diseases	Increases anti-inflammatory activity by inhibiting TNF-α, NO, and MPO activity and significantly decreases their production in activated human neutrophil cells.	[43]

Uses of Rutin	Therapeutic Agent	Cell Lines and Assays Used	Outcomes	Reference
	Rutin microencapsulated in chitosan matrix	NCTC-2544 and C-28 cells	Increased *in vitro* anti-inflammatory activity as compared to free active compound.	[44]
	Rutin combination with paracetamol and NSAIDs (diclofenac)	RAW 264.7 macrophages	Synergetic effects of rutin with both drugs.	[45]
Antioxidant	Rutin	Neurodegenerative diseases	Reduced proinflammatory cytokines, increased oxidative enzymes, induced mitosis, and reduced mRNA expression.	[14]
	Rutin with ascorbic acid	UVA and UVB exposed human skin keratinocytes and fibroblasts	Increased activity of rutin when given in combination with ascorbic acid.	[46]
	Chitosan/silver nanostructures of rutin	DPPH, *Bacillus subtilis, Escherichia coli,* Methylene blue dye degradation	Increased antioxidant activity of rutin with a corresponding increase in the concentration of chitosan nanostructures.	[47]
	Rutin and quercetin	*In vitro* binding of neratinib with human serum albumin.	Both bind with human serum albumin and displace it.	[48]

Uses of Rutin	Therapeutic Agent	Cell Lines and Assays Used	Outcomes	Reference
	Nano lipid complex of rutin	*In vitro* and *in vivo* methods	Increased solubility and bioavilability along with enhanced hepatoprotectiv e and antioxidant effects of nano complexes.	[49]
	Rutin	Cisplatin-induced ovarian damage	Stores normal percentage of follicles and cell proliferation, decreases apoptosis and reactive oxygen species, increases active mitochondria and glutathione levels along with enhanced p PTEN and reduced p FOXO3a.	[50]
Cardioprotective	Rutin	H9c2 cells qRT-PCR and western blot method	Enhanced SIRT1 expression, lactate dehydrogenase, creatine kinase-MB, and aspartate transaminase with reduced apoptotic rate and caspase 3 activity.	[51]
	Rutin	Cardiac and erythrocyte membranes	Increased erythrocyte Na/K ATPase activity with	[52]

Uses of Rutin	Therapeutic Agent	Cell Lines and Assays Used	Outcomes	Reference
			reduced Ca ATPase activity and cardiac Na/K ATPase activity.	
	Rutin	Streptozotocin induced diabetes	Reduced degenerative alterations of heart with improved ECG.	[53]
	Rutin	Sodium fluoride induced hypertension	Reduced kidney injury molecule I and nuclear factor kappa beta, increased nitric oxide, and normalized blood pressure.	[54]
	Rutin	Cardiac remodelling	Triggered SIRT1/NRF2, reduced apoptosis, autophagy oxidative stress, and cardiac fibrosis.	[54]
Neuroprotective	Rutin with selenium	3-nitropropionic acid induced huntingtons' diseases	Co-administration reduced oxidative stress, apoptotic cascade, and inflammation of neurons, as well as reduced astrocyte activation and enhanced neurotrophic factors.	[55]
	Rutin	Brain slices	Reduced cell death and loss	[56]

Uses of Rutin	Therapeutic Agent	Cell Lines and Assays Used	Outcomes	Reference
			of glutamate synthase. Increased glutamate uptake in cerebral cortex.	
	Rutin	Endovascular perforation method	Reduced levels of RAGE, NF-kB, and inflammatory cytokines.	[57]
	Rutin	*Caenorhabditis elegans* Dye filling assay	ASH neurons maintained by rutin and reduced degeneration.	[58]
	Rutin	Colistin induced oxidative stress, inflammation, and apoptosis in brain	Reduces colistin-induced alterations and rehabilitate brain function.	[59]
	Rutin	Spinal cord injury	Reduced NLRP3, ASC, IL-1 beta, IL18, TNF alpha, ROS, and histologic changes, along with enhanced locomotion.	[60]
Antimicrobial	10 flavonoids including rutin, kaempferol, luteolin, quercetin, apigenin, hesperidin, sinensetin, naringenin and 3,5,6,7,8,3',4'-	DPPH assay	Potential activity showed by rutin against *Klebsiellapnemoniae*.	[61]

Uses of Rutin	Therapeutic Agent	Cell Lines and Assays Used	Outcomes	Reference
	heptamethoxyflavone			
	Polycaprolactone film of chitosan and rutin	Electrospinning	Formulated film. Showed increased antimicrobial and antioxidant activity.	[62]
	Pure and encapsulated rutin	*Streptococcus pyogenes, Enterococcus faecalis, Bacillus cereus, Pseudomonas aeroginosa, Klebsiella*	Encapsulated rutin showed increased, decreased, or constant antimicrobial activity depending upon strain type.	[63]

2.4. Cardioprotective Property

Yang H et al analyzed the reduction effect of hypoxia-induced myocardial injury via the upregulation of SIRT1 expression of rutin. The objective was to explain the cardioprotective effects of rutin in myocardial injury induced by hypoxia and to evaluate the mechanism involved. For this purpose, qRT-PCR and western blot methods were used to perceive SIRT1 (Silent Information Regulator 1) expression. H9c2 cells were given 50 micro molar rutin or merged with SIRT1 inhibitor for an hour, then exposed to hypoxia for 6 hours and kept at reoxygenation for 24 hours. The results indicated that both in H9c2 cells and hypoxia/reoxygenation stimulated H9c2 cells, rutin increased the expression of SIRT1. Rutin also enhanced the viability of hypoxia/reoxygenation-exposed H9c2 cells. Increased levels of LDH, CK-MB, and AST acted as the indicators of hypoxia/reoxygenation-induced myocardial injury, which were eradicated by rutin. Furthermore, rutin also caused a reduction in apoptotic activity and caspase-3 activity induced by hypoxia (reoxygenation). In addition, rutin overrode the hypoxia/reoxygenation-induced reduction in catalase, glutathione peroxidase, and superoxide dismutase activities and increased malondialdehyde. These cardioprotective effects of rutin were terminated

by SIRT1. Thus, rutin was found to be effective in the treatment of hypoxia/reoxygenation-induced myocardial injury [51].

Oluranti OI et al assessed the role of rutin in enhancing the activity of erythrocyte membrane-bound ATPase and cardiac membranes in male rats upon exposure to lead acetate and cadmium chloride. They analyzed rutin's effect on cardiac and erythrocyte disorders due to the presence of cadmium (Cd) and lead (Pb) in male rats. A total of 25 male rats were selected and different groups were made which were given 60mg/kg P.O Pb, 5mg/kg P.O Cd, rutin, Pb, and Cd (50mg/kg rutin, 60mg/kg Pb, 5mg/kg Cd P.O), respectively. In cardiac and erythrocyte membranes, the activity of Na/K ATPase along with calcium was observed. The results indicated an increase in Na/K activity in Pb treated group (172%) and a decrease in activity in Cd treated group (33.7%). When Pb and Cd groups were compared, rutin enhanced the erythrocyte Na/K ATPase activity of these groups. Further, there was a reduction in erythrocyte calcium-ATPase activity in Pb, Cd, and Cd + Pb groups (68%, 68% and 55.3%), respectively. Whereas, there was no change in Na/K ATPase activity of cardiac in Pb and Cd groups, although reduced activity was observed in the Cd + Pb group. Thus, rutin overrides the altered cardiac and erythrocyte membrane-bound ATPase activity caused by exposure to Cd and Pb [52].

Saklani R et al performed a study on diabetic rats (streptozotocin induced) to investigate the cardioprotective effect of rutin, as well as its antioxidant effect. The study was performed on 4-week-old diabetic rats for a duration of 24 weeks in order to imitate the cardiotoxic effect of hyperglycemia. It examined the effect of 50mg/kg/day of rutin in improving its cardioprotective effects. Experimental rats showed changes in ECG, a lowered antioxidant ability, and enhanced inflammatory strike, along with degenerative alterations. However, these effects were reduced by treatment with rutin along with a decreased blood sugar level, decreased HbA1c, and decreased TNF-α expression, as compared to the control group. Moreover, rutin also provided protection from oxidative stress related to diabetes and caused a reduction in degenerative alterations in the heart, along with an improved ECG in the rutin-treated group. The results indicated that diabetic cardiomyopathy is due to oxidative stress and inflammation, whereas rutin is effective in the treatment of diabetic cardiomyopathy due to its potential biological properties [53].

Ademola and colleagues induced hypertension in rats by giving them sodium fluoride (NaF) and studied rutin's effect for its treatment. A total of 40 albino rats were selected and divided into 4 groups. Group A was considered as control and given normal saline, while group B was provided 300ppm NaF via drinking water. Furthermore, groups C and D were given 100mg/kg NaF along with 200mg/kg rutin by mouth daily for a duration of one week. The results indicated that exposure to NaF only caused increased blood pressure along with a reduction in serum nitric oxide. Upon immune histochemical examination, group B showed increased expression of kidney injury markers. Whereas, the rats exposed to NaF along with rutin showed normal blood pressure, reduced kidney injury, as well as enhanced bioavailability of nitric oxide. Thus, rutin proved to be effective in NaF-induced hypertension [54].

Siti HN et al explained rutin's functions in cardiac remodeling. Rutin produces cardioprotective effect during cardiac remodeling and is very effective as a remedial in cardiac pathology, especially heart failure. Recent studies show that in cardiac remodeling pathogenesis, rutin causes molecular and cellular alterations. It plays a cardioprotective role due to triggering SIRT1/NRF2 pathways, reduction of apoptosis and autophagy, as well as decreasing oxidative stress. In diabetic rats with cardiac-myopathy, rutin was given at 50mg/kg/day dose orally for 24 days, resulting in the reduction of TNF-α and CRP levels. Rutin reduces cardiac fibrosis by causing reduction in growth factors and expression of MMP. Thus, rutin is effective in the reduction of pathological cardiac remodeling [11].

2.5. Neuroprotective Property

Abdel Fattah MS et al performed a study to analyze the co-administration of selenium and rutin to assess their effectiveness in treating Huntington's disease (HD) on mouse models. The basic aim was to investigate the neuroprotective effect of the co-administration of rutin and selenium in mice having 3-nitropropionic acid-induced HD-like symptoms. For 30 days, rutin and selenium nitrate were administered to mice orally, 50 mg/kg and 0.2 mg/kg daily, respectively. From 8^{th} to 21^{st} day, the animals were injected with normal saline intraperitoneally after 1 hour. It caused alterations in redox reaction showed by enhanced striatal malondialdehyde and nitric oxide levels, along with reduced levels of antioxidants including catalase, superoxide dismutase, glutathione reductase, and glutathione. Further, inflammation was increased by the production of interleukin-1beta,

TNF-alpha, and myeloperoxidase. In the striatum, a pro-apoptotic cascade was also present. The co-administration of rutin and selenium showed neuroprotective activity via blocking weight loss, oxidative stress, inflammation of neurons, as well as an apoptotic cascade. Rutin and selenium prevented astrocytes production, enhanced neurotrophic factors derived from the brain, and caused an increase in cholinergic and monoaminergic transmission caused by 3-nitropropionic acid. Thus, the co-administration of selenium with rutin showed effectiveness against Huntington's disease due to its neuroprotective, anti-inflammatory, antiapoptotic, and antioxidant roles [55].

Ferreira RS et al performed a study on rat brain slices to investigate the neuroprotective action of rutin. The objective was to determine the mechanism through which rutin performs neuroprotective activity. They assumed that the increased metabolism of glutamate in astrocytes is the underlying mechanism. In order to investigate this assumption, they used post-natal Wister rats and treated them with rutin. Then, they determined the number of proteins associated with glutamate metabolism and neuroprotective properties of rutin. To determine glutamate uptake, they used the cerebral cortex of adult Wister rats. The results indicated that in post-natal Wister rats, rutin prevented cell death as well as the loss of glutamate synthase caused by glutamate linked with increased glutamate aspartate transporter in their brain. Moreover, in the cerebral cortex of the adult Wister rats, rutin caused an increase in glutamate uptake. Thus, rutin is an effective neuroprotective agent as it inhibits glutamate excitotoxicity. The underlying mechanism remains the maintenance of glutamate metabolism in astrocytes [56].

Hao G et al performed a study to examine the neuroprotective effect of rutin on rats suffering with a subarachnoid hemorrhage. The aim was to determine the effect of rutin on neuro-inflammation and the mechanism of subarachnoid hemorrhage. The endovascular perforation method was used. Adult male rats were selected and divided into 3 groups. One was a sham group. Others included the subarachnoid hemorrhage+vehicle group and subarachnoid hemorrhage+rutin group (50 mg/kg), which were intraperitoneally (i.p.) administered 30 minutes after subarachnoid haemorrhage. After 24 hours, rats were examined for neurologic scores, the permeability of BBB, and brain water content along with cell death in the brain (in the cerebral cortex). Inflammation in the brain was determined by

assessing the levels of NF-KB, RAGE, and inflammatory cytokines. The results revealed reduction in the levels of RAGE, NF-κB, and inflammation cytokines, along with reduction in secondary brain cell injury [57].

Cordeiro LM et al performed a study on the *Caenorhabditis elegans* model of HD to investigate the neuroprotective effect of rutin. Rutin solution was made by dissolving it in absolute ethanol. Later, it was added to agar plates containing *E. Coli* OP50 in nematode growth medium to obtain final concentrations of 15, 30, 60, and 120 μM (9.16–73.26 mg rutin/ml agar) and 1% ethanol. The aim was to investigate the neuroprotective effect of rutin via ASH neurons and antioxidant action. The dye-filling assay method was used. Behavioural alterations, neuronal polyQ aggregates, and degeneration were examined. The study concluded that the long-term use of rutin helps in the maintenance of ASH neurons and also reduces degeneration [58].

Celik H et al studied the neuroprotective activity of rutin to treat colistin-related apoptosis, inflammation, and oxidative stress in rat brains. A total of 35 male rats were selected and divided into five groups namely control group, rutin group (which received 100 mg/kg of rutin), colistin group (administered with 15 mg/kg colistin), colistin-rutin group (provided with 15 mg/kg of colistin and 50 mg/kg of rutin), and the colistin-rutin-100 group (administered with 15 mg/kg of colistin and orally received 100 mg/kg of rutin). The administration of colistin caused an increase in glial fibrillary acidic protein and the neurotrophic factor derived from the brain, as well as acetylcholinesterase and butyrylcholinesterase action. Moreover, it caused a reduction in cAMP activity and kinases 1 and 2 signal, regulated extracellularly. It also caused oxidative ruination and increased the apoptotic and inflammatory factors. The results showed that rutin reinstated brain function by reducing all the colistin-induced alterations. Thus, rutin proved to be effective in colistin-induced neurotoxicity [59].

Wu J et al studied the properties of rutin in neuro-inflammation of spinal cord injury in rats.. A total of 120 female rats were chosen and divided into four groups (sham group, spinal cord injury group, spinal cord injury+rutin50mg group, and spinal cord injury+rutin100mg group). The effect of rutin on inflammatory markers, histological changes, and locomotion scale was examined. The results showed that spinal cord injury caused an increase in NLRP3, ASC, IL-1beta, IL-18 and TNF-alpha. Whereas, rutin caused a decrease in their levels and reduced reactive oxygen

species and histological changes, while enhancing locomotion. Thus, rutin was found to be effective in the neuro-inflammation of spinal cord injury because of its anti-inflammatory and antioxidant activity [60]. Figure 3 illustrates anticancer, anti-inflammatory, antioxidant, cardioprotective, neuroprotective, and antimicrobial effects of rutin.

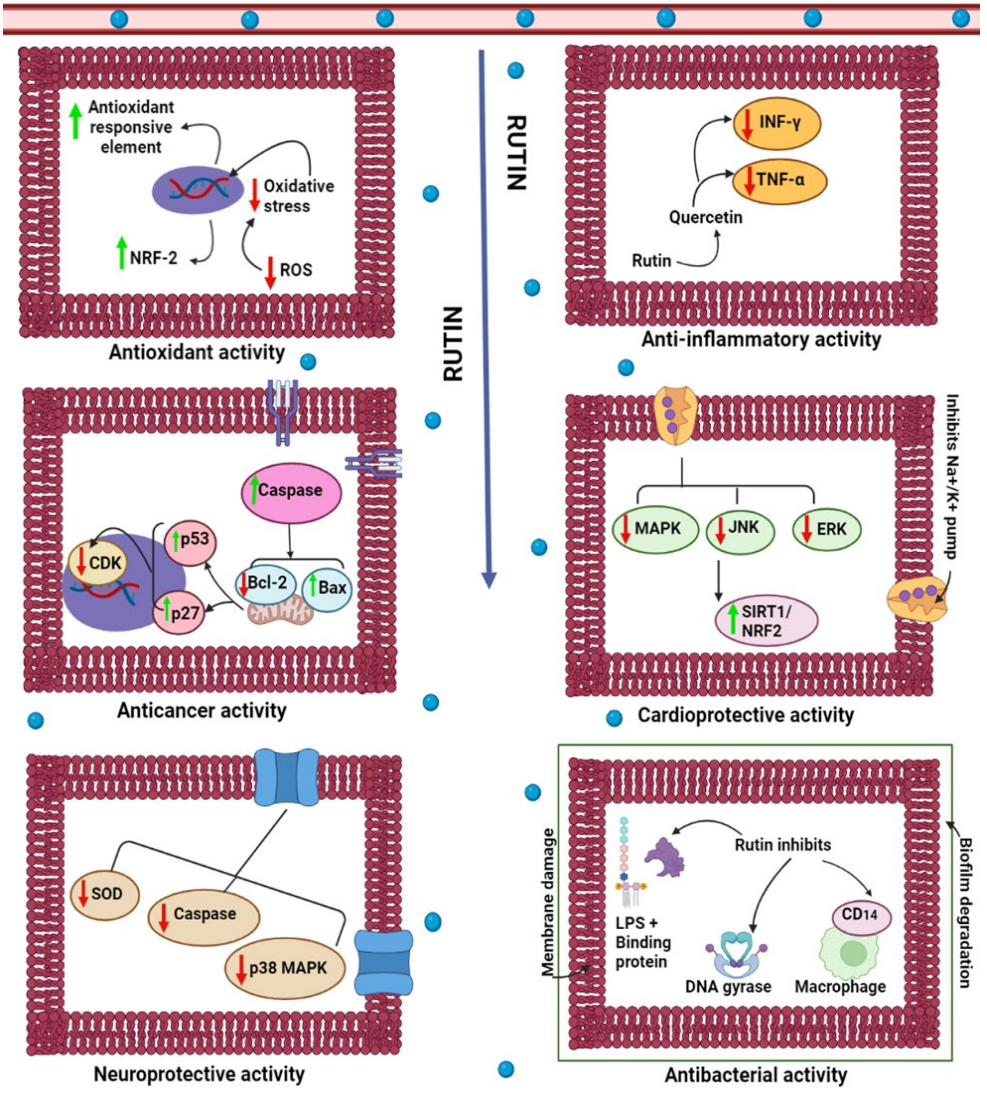

Figure 3. Cellular Mechanisms of Rutin as Antioxidant, Anti-Inflammatory, Anticancer, Cardioprotective, Neuroprotective, and Antibacterial Agent

2.6. Antimicrobial Property

Wang Z et al performed a study on 10 flavonoids for their antimicrobial and antioxidant effects by using DPPH assay. Two-fold serial dilutions were prepared separately of 10 flavonoids including rutin to perform the antibacterial analysis. The results showed that rutin has the highest antioxidant activity as compared to kaempferol, luteolin, quercetin, apigenin, hesperidin, sinensetin, naringenin, naringin, and 3,5,6,7,8,3′,4′-heptamethoxyflavone. The study also revealed that rutin has a potential antibiofilm effect against *Klebsiella pnemoniae.* Further, it also has a strong antimicrobial effect as well as a strong inhibitory effect against *K. pnemoniae.* It is very beneficial because multidrug resistance (MDR) and extended drug resistance (XDR) in *K. pneumoniae* emerge from the use of conventional antibiotics. MIC of rutin against *K. pnemoniae* ATCC700603 was 1024 micrograms per ml, while *E. coli* ATCC25922 was 512 micrograms/ml. Rutin inhibited the biofilm production along with the growth of the bacteria [61].

Piri H et al carried out a study to formulate a novel film of polycaprolactone in which chitosan and rutin were incorporated. Electro-spinning method was used to formulate the films of PCL, PCL-chitosan, PCL-rutin, and PCL-chitosan-rutin (PCL-CS-R). These films were investigated for their physical properties, *in vitro* antibacterial and antioxidant effects, as well as their antibacterial effect on rainbow trout packaging. The results indicated that PCL-CS-R film could be used in intelligent packaging, since it has potent antimicrobial and antioxidant effects [62].

Danciu C et al conducted a study to check the antiproliferative and antimicrobial effects of pure, encapsulated rutin and beta-cyclodextrin complexes of rutin. Antimicrobial screening showed that rutin has an antimicrobial effect against *Streptococcus pyogenes, Enterococcus faecalis, Bacillus cereus, Pseudomonas aeroginosa, Klebsiella pneumoniae,* and *Escherichia coli.* On the other hand, rutin showed little activity against *streptococcus pneumoniae* and *staphylococcus aureus.* The study indicated that when rutin was mixed with cyclodextrin, antibacterial effect increased depending upon the type of the selected strain [63].

4. CONCLUSION

From the above summarized work, it is concluded that rutin, a natural flavonoid, is effective as an anticancer, anti-inflammatory, antioxidant, cardioprotective, neuroprotective, and antimicrobial agent. These effects are produced by rutin alone or in combination with other agents. For example, rutin and ferulic acid have an anti-inflammatory effect. Furthermore, the combination of rutin with ascorbic acid and quercetin has an antioxidant effect, while rutin and selenium have neuroprotective effects. In addition, rutin also has anti-SARS-CoV-2 activity. It can be formulated in different formulations such as chitosan/silver nanostructures, nano-crystals, and microencapsulated in chitosan matrix for enhanced efficacy. The main problem in the use of rutin is its solubility and bioavailability, which was overcome in this study by formulating its nano-lipid complexes. However, rutin shows synergistic effects with NSAIDs. Further, there is a need to establish the pharmacokinetic and pharmacodynamic profile of various combinations of rutin to determine its half-life, Cmax, and Tmax with the aim to evaluate its steady state concentration and toxicity, to develop its different dosage types, and to understand its multi-purpose usage.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting this study is available within the article and supplementary material. Raw data of the original studies can be obtained from the corresponding author upon request.

FUNDING DETAILS

No funding has been received for this work.

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