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Review

Biological and Functional Properties of Wedelolactone in Human Chronic Diseases

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Abstract: Medicinal herbs are well known and studied over the past millennia in most of the developing countries as a rational means of treatment against various diseases and disorders. Wedelolactone (WDL), a major bioactive compound in *Eclipta prostrata* L (*Eclipta alba* L), has been reported with potential benefits in human health against chronic diseases. However, a comprehensive study on WDL pharmacological benefits in various ailments, to the best of our knowledge, is not yet reported. Thereof, the present review provides the recent therapeutic applications in reference to biological and functional activities against major human chronic diseases, including cardiovascular, cancer, diabetes mellitus, liver disease, Alzheimer's disease, and androgenetic alopecia. In this study, we collected all the relevant experimental information on WDL from Scientific databases such as PubMed, Web of Science, Science Direct, and Google Scholar. Conclusively, WDL is recognized as a key anti-oxidant with both specific regulator and inhibitor of major drug targetable proteins in human chronic diseases and disorders. Hence, WDL as a novel therapeutic bioactive molecule is advised to explore further for relevant pharmacological activities.

Keywords: Wedelolactone; *Eclipta alba* L; *Ecliptaprostrata* L; bioactivity; bioavailability

1 Introduction

The natural sources of plant food products, such as whole grains, berries, fruits, vegetables, and bioactive non-nutrient plant compounds called phytochemicals are intensively researched for their possible use in drug discovery and improvement. The use of these natural compounds have gained popularity in nutraceutical and pharmacological purposes due to their potential benefits and safety [1–4].

Currently, medicinal plants are widely used as traditional and preventive medicine, especially in the treatment of metabolic disorders [5,6].

Polyphenols are secondary plant metabolites that act to defend the plants against ultraviolet radiation, oxidants, and pathogens [7,8]. These natural phytochemicals are abundantly found in vegetables, fruits, cereals, whole grains, legumes, coffee, and cocoa [7]. Structurally, polyphenols are classified into numerous classes based on the number and arrangement of phenolic rings. Among them, flavonoids are the major abundant class of polyphenols in the human diet and more than 4,000 types of polyphenols have been identified [9,10]. In general, these plant polyphenols promote health by their powerful antioxidant properties.

The main structural feature of phytochemicals in plants is the presence of hydroxyl groups attached to one or more aromatic rings. Plant polyphenols are classified into two major groups: Flavonoids and non-flavonoids. Flavonoids have a common flavone core consisting of 15 carbon atoms, which is further divided into flavonols, flavone-3-ols, anthocyanidins, flavones, and chalcones. Non-flavonoids have an aromatic ring with one or more hydroxyl groups. These included stilbenes, phenolic acids, saponin and other polyphenols like tannin and curcumin. It has been estimated that dietary intake of polyphenols is approximately 1.2 g/day [11]. Among the various interesting biological properties exhibited by plant polyphenols, their potential against various diseases have attracted much attention.

Among many phytochemicals, coumestan, known as 6H-benzofouro[3,2-c][1]benzopyran-6-one, represents the basic ring system for a number of naturally occurring wedelolactone (WDL) and many other compounds with antibacterial, antifungal, antimyotoxic, phytoestrogen, and phytoalexin activities [12]. Many of their biological activities can be attributed to their action as phytoestrogens. Further, polyphenols have shown beneficial effects in the experimental studies as edible health-promoting supplements [13].

In this review article, we describe the therapeutic potential and biological activities of WDL with underlying molecular mechanisms by citing available scientific reports. We also discuss the limitations and possibilities of WDL in pharmaceutical and clinical development. In addition, the structural changes, physico-chemical, pharmacokinetics, drug delivery and evaluation characteristics of WDL have been discussed.

2 Material and Methods

For relevant literature an internet search was conducted using PubMed, Web of Science, Science Direct and Google scholar typing the keywords "*Eclipta prostrata* L" OR "*Eclipta alba* L" AND "Wedelolactone-antioxidant, antidiabetic, anticancer, antimicrobial, cardiovascular, hepatoprotective, osteoporosis, anti-hair loss and neurotoxicity" in title/abstract/keywords, without date restriction to retrieve the majority of the research articles (*in vitro*, *in vivo* and clinical).

3 Wedelolactone

3.1 Plants

Wedelolactone and dimethyl wedelolactone (DWL) are the coumestans present in *Eclipta prostrata* L (Fig. 1A) (synonym: *Eclipta alba* L. "Bhringaraj"). The plant belonging to the family Asteraceae is a small, branched annual herb inhabiting at tropical and subtropical regions of the world with white flower heads. *E. prostrata* is one of the oriental herbs known as 'Hanryoncho' in Korea and China. The plant grows abundantly in cool, humid locations throughout India and Northern Asia [14]. *E. prostrata*, a traditional herbal medicinal plant, has been a long used in Asia and South America for the treatment of various human diseases [15].

3.2 Chemistry

Wedelolactone (Fig. 1B), a natural coumestan was first obtained from the *Wedeliacalandulacea* extract in 1956 and later isolated from Bhringaraj (*Eclipta alba* L.) which is an acrid, bitter ethno-medicine used

widely for dermatological and trichological health as well as in the treatment of cirrhosis, infective hepatitis, and hepatic enlargement.

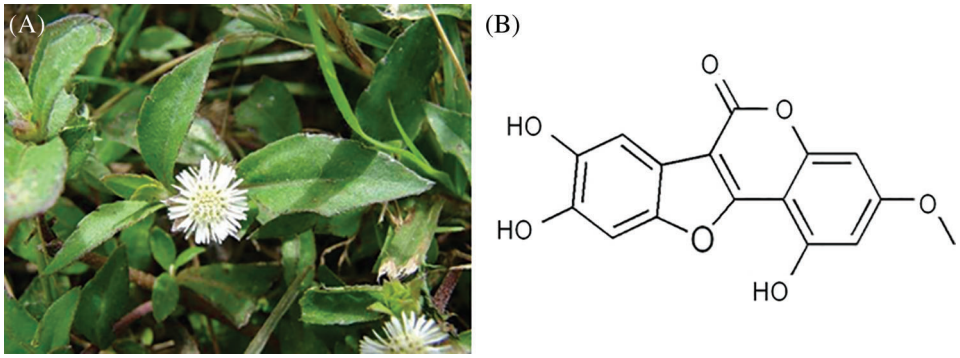

Figure 1: *Eclipta alba* L. (A), and structure of wedelolactone (B)

Chemical name: 7-methoxy 5,11,12-trihydroxy-coumestan

Chemical formula: $C_{16}H_{10}O_7$

Molar mass: 314.249 g/Mol

Boiling point: 498.4°C at 760 mmHg

Density: 1.655 g/cm^3

Pubmed CID: 5281813

3.3 Extraction Methods of the WDL

3.3.1 Solvent or Soxhlet Extraction Method

Shanshol et al. [16] used a Soxhlet apparatus to extract WDL from aerial parts of *E. alba*. The shade dried plant material powder was used to extract WDL. Various solvents such as water, hexane, absolute methanol, and absolute ethanol were used at 50°C for 36 h to extract the material. The extracted material was filtered and concentrated using a rotary evaporator and the dried material was kept at 4°C until further use. The extracted sample was also evaluated for different phytochemical constituents. Patil et al. [17] also used this method for extracting WDL from *W. calendulacea* using methanol as a solvent for 24 h and dried using a rotary vacuum evaporator.

3.3.2 Ultrasound-Assisted Extraction

In this method, asonochemical reactor consists of an ultrasonic probe was employed to extract WDL from *E. prostrata*. Response surface methodology and central composite design were used to optimize the process parameters [18]. The important parameters for optimizing the process were type of solvent, temperature, extraction time, solvent to material ratio, ultrasonic power and extraction time. In the process, the powdered material (screened from #80-100 standard filter) was soaked in the solvent for about an hour in a vessel and ultrasonication was performed with 50% duty cycle at 5 s ON and 5 s OFF cycle by dipping 1.0 cm probe into the solvent. Savita et al. [19] used this method to extract WDL from *E. alba* with 1:5 ratio of methanol, herb powder, and found 0.36% yield of WDL.

3.3.3 Maceration Followed by Percolation

In this method, air-dried and coarse powder of whole plant of *E. alba* was used and macerated for 24 h in methanol and then percolated in a vessel until a colorless percolate was obtained [19]. The percolated extract was dried using a vacuum rotary evaporator at 40°C and obtained a green color sticky mass. The isolation of

WDL was done through column chromatography using 60–120 mesh silica gel and toluene as an eluting solvent. The isolated material was further purified by preparative high-performance thin layer chromatography and found 0.38% yield. Finally, it was characterized and confirmed by spectroscopic techniques.

3.3.4 Supercritical CO_2 Extraction

Patil et al. [17] extracted WDL from *W. calendulacea* using supercritical CO_2 method. In this method, the extraction column was filled with sample powder and fixed in a column oven. A chiller unit was used to pass CO_2 to achieve the desired working pressure. A solvent pump was employed to introduce methanol. The whole system was thermostatically maintained and then six port valves were opened to pass the supercritical CO_2 to start the extraction cycle. The first cycle was static to achieve complete contact with sample, while the second cycle was dynamic, in which steady flow of CO_2 to sample was achieved. The extract was collected in a vessel and analyzed to confirm WDL. They found that the concentration of WDL decreased when the pressure and temperature were increased from 25.0 to 35 MPa and 40 to 80°C, respectively. Similarly, when the extraction time was increased from 30 to 90 mins, the concentration of WDL was found to increase [17]. Also, Savita et al. [19] extracted 0.002–0.013% WDL using supercritical fluid extraction at different pressure (4000–6000 psi) and temperature (40–50°C) with a CO_2 flow rate of 23.98 mL/min from *E. alba*.

3.3.5 Microwave-Assisted Extraction

Dang et al. [20,21] used a reflux system equipped with round bottom flask to extract WDL from *E. alba*. In their procedure, 90% ethanol was used as an extraction solvent with 200W microwave radiation for 30 mins. Then, extracted mixture was filtered and the process was repeated with the same sample three times. Afterward, the extract was dried using a rotary vacuum evaporator at 50°C, the dried extract was dissolved in hot water and filtered. The aqueous solution was extracted by ethyl acetate for three times, dehydrated over anhydrous sodium sulfate and finally 80%–90% solvent of the solvent was evaporated at 50°C under vacuum. Then, a silica gel column was used to purify WDL with dichloromethane, methanol and small amount of acetic acid as mobile phase. Final purification was performed using dilution crystallization with single factor analysis and response surface methodology. This resulted in 77.66% yield and 99.46% purity of WDL [21].

3.3.6 Ultra-High Pressure-Assisted Extraction

In this method, high-speed counter-current chromatography was employed with ultra-high-pressure extraction (UHPE) to extract and purify isodemethyl-WDL and WDL from *E. alba* [22]. The crude powdered herb was sieved using 60–80 mesh, filled into a polyethylene bag with the extraction solvent, sealed and placed in an ultra-high-pressure vessel for extraction. Then, the extract was centrifuged for 5 mins at 6000 rpm and filtered through 0.45 micron membrane filter. The WDL content was determined using high performance liquid chromatography. Afterward, two phase solvent system, i.e., petroleum ether-ethyl acetate-methanol-water in the ratio of 3:7:5:5 was used for high speed counter current chromatographic separation. By this method, 300 mg of crude extract yielded 23.5 mg WDL, 6.8 mg isodemethyl-WDL and 5.5 mg luteolin with more than 95% purity in a one-step separation. Various UHPE parameters such as time, solvent, pressure and solid-liquid ratio were optimized using orthogonal array. The optimum conditions were found to be 80% aqueous methanol, 3 mins extraction time, 1:20 solid-liquid ratio and 200 MPa pressure for good yield.

3.3.7 Aqueous Two-Phase System (ATPS) Extraction Method

In this method, ATPS was prepared from various mol. wt. of polyethylene glycol (PEG) and sodium citrate salt [23]. A fixed amount of *E. alba* dried powder was taken in a conical flask at a specified pH. Then, the mixture was agitated at a temperature of 30 ± 2°C and 600 rpm for 2 h using a magnetic stirrer. Afterward, the mixture was centrifuged at 8000 g rpm for 10 mins, the resultant mixture was collected

using a separating funnel and analyzed by high performance liquid chromatography. It was observed that as the concentration of sodium citrate, mol. wt. of PEG, concentration of PEG and pH were increased from 14% to 16%, 4000 to 6000, 12% to 18% w/v and 5 to 7, respectively, the yield was found to increase, whereas, further increase in the range of parameters resulted in a decreased yield [23]. The optimized parameters for ATPS system were PEG 6000, PEG concentration 18% (w/v), salt concentration 17.96% (w/v), and pH 7 to get an extraction yield of 6.73 mg/g [23].

3.4 Bioavailability

Pharmacokinetic studies involving albino rats indicated that WDL administration with paracetamol did not alter the bioavailability of paracetamol in plasma. The UV λ_{max} for WDL was found to be 351, 248, and 208 nm in the spectrometric assessment. The following are the physical and chemical properties observed by WDL. Melting point: 310°C, retention time (HPLC):11 mins. There was no change in C_{max} between paracetamol and WDL, but the T_{max} of paracetamol was 2 h in control group rats, while WDL oral treatment with paracetamol resulted in a shift of T_{max} to 2 to 3 h without substantially affecting the area under the curve [24]. A validation study showed that inter- and intra-day accuracy of quality control WDL specimens was within the acceptance threshold (85%–115 %). Mean recovery was found to be within the acceptable limit of >95% (98.8% WDL) for marker quality control samples. It was found that WDL was stable at ambient temperature for 6 hours and below 8°C for 15 days [25]. In a quantification study of WDL in mice plasma, an apparent distribution of 53.5 L/kg and a clearance of 6.39 L/h/kg were observed. In addition, the area under the conc-time curves AUC/0-24 for WDL was 27.5 ng/h/mL. The concentration of plasma above the lower limit of quantitation was noted up to 24 h showed 10.54 h of Mean Residence Time (MRT0-inf) for WDL, which may be the reason for its sustained pharmacological activity [26]. Chen et al. [27] studied the pharmacokinetic of WDL in rats and found that 5.00 mg/kg WDL was rapidly absorbed through gastrointestinal tract. This study provided a theoretical basis for pharmacological research on pre-clinical medicine.

4 Therapeutic Application of WDL

4.1 Free Radical Scavenging Activity

Free radicals are extremely reactive molecular species with an unpaired electron in the exterior valence orbitals that causes them to capture electrons from other substances to neutralize themselves. It has been known that the overproduction of free radicals, including reactive oxygen and nitrogen species (RONS) play a key role in the development of many chronic diseases. It has been experimentally demonstrated that WDL extracted from *E. alba* has antioxidant potency to scavenge nitric oxide radical/superoxide [28]. The mechanistic investigations showed single electron transfer to be the primary pathway and Fe^{2+} chelation is a secondary pathway. Both of these pathways can be assigned to the catechol moiety of WDL rather than the cumestane skeleton [29].

4.2 WDL as an Effective Antimicrobial Agent

In recent years, phytochemicals with antimicrobial properties have gained overwhelming attention pharmaceutical applications. *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Salmonella typhimurium* are the most vulnerable species to the antimicrobial activity of WDL. The most resistant strain of bacteria was *Shigella flexneri*. These finding indicated that WDL is a promising antimicrobial agent [30]. It has been experimentally demonstrated that crude extract of *E. alba* has antimicrobial, antibacterial and antiviral activities [31–33]. More recently, it has been demonstrated that WDL showed excellent activity against *Salmonella typhimurium* and *Staphylococcus epidermidis*. The minimum inhibitory concentration (MIC) and zone of inhibition of *Staphylococcus epidermidis* and *S. Typhimurium* were 15 µg/ml; 10.3 mm and 25 µg/ml; 9.2 mm, respectively. *E. coli* was a highly resistant bacteria strain [34].

4.3 Anti-Cancer Effect of WDL

Cancer is one of the world's main health issues and is the second leading cause of death following heart disease [35]. The latest report released by the world health organization shows that 8.8 million individuals died in 2015 as a result of cancer, which accounts for around 16% of the world's total fatalities. WDL has been shown to exhibit significant decreases in the protein values, nuclear accumulation, DNA binding and transcriptional activities of c-Myc. It can efficiently down-regulate the oncogenic role of cancer cells in c-Myc, that can be a new route to develop a therapeutic approach for Myc-driven prostate cancer [36]. In addition, WDL can specifically inhibit the estrogen receptor (ER) signaling and block the 17β-estradiol (E2) activated cell proliferation in the estrogen cancers [37]. The results of the study found that WDL specifically enhanced interferons (IFN-α)-induced signal transducers and activators of transcription 1 (STAT1) phosphatase by inhibition of STAT1 phosphatase T-cell protein tyrosine phosphatase (TCPTP) and possibly interacted with the c-terminal auto-inhibitory domain of TCPTP, to treat apoptosis of cancer cells [38]. The release quantity of liposomal indocyanine green (Lip-ICG) WDL reached upto 96.7% at 8 h, the release of drug on site was achieved under NIR irradiation. In addition, Lip-ICG WDL under near infrared (NIR) clearly inhibited the development of HepG2 cells, and early stage apoptosis in Hep G2 cell lines of rats were 33.7 percent. In addition, the tumor mice treated with the medication were inhibited to 81% [39]. It has been evaluated that WDL can destroy breast cancer cells, mediated by proteasomal protein targets (p21,27,53 and Bax) mortification pathway [40]. The results also suggested that WDL triggers selectivity induced caspase-3 protein apoptosis cells in prostate cancer *via* molecular mechanism involving the downregulation of protein kinase [41]. A recent study evaluated the *in vivo* effect of WDL (30 μg/ml) played an important role for regulation of zymosan induced inflammation signals in bone marrow-derived macrophages (BMs), mediated by zymosan secretion of tumor necrosis factor-α (TNF-α), interleukin-1, interleukin-6, interleukin-12 but not interleukin-10. Furthermore, decreased levels of superoxide generation, NADPH oxidase, phosphorylation of p47phox in BMDMs by pre-treatment of WDL have been demonstrated [42]. Another interesting study has found that WDL had a promisingaction in suppressing early tumor promotion events triggered by ultraviolet (UV) B radiation exposure as shown by ornithine decarboxylase, vimentin and vascular-endothelial growth factor expression [43]. Peng et al. [44] investigated the effect of WDL suppression in the proliferationof cell nuclear antigen and melanoma cell MV3 cell cycle proteins through AMP-activated protein kinase and Akt signal pathway. It has also been reported that WDL-coated poly(lactic-co-glycolic acid)-nanoparticles (PLGA-NPs) restricted the transition of epithelial cells to mesenchymal cells thereby preventing the cells from migrating and invading and reduced the growth of breast cancer stem cells in MDA-MB-231 cells [45]. Recently, Zhang et al. [46] showed that a combinational therapeutic approach (chemo photothermal) using WDL liposome coated gold nano-shells inhibits the tumor cells upon NIR irradiation andpromotes targeted drug release. The hyperthermia effect of gold nanoshells enhanced the release of drug onto the targeted site inhibited the 143B tumor cells up to 95.73%. In another study, WDL was shown to inhibit the protein cyclin D and increase p21 expression protein. Also, WDL inhibited activation of Akt but triggered activation of AMPK [47].

4.4 Antidiabetic Effect of WDL

Diabetes is generally characterized by hyperglycemia. In the past decade, it has been proved that WDL has good antioxidant and antiglycation activities. The significantly decreased levels of glycosylated haemoglobin (HbA1c) and glycated serum proteins, indicated the low generation of advanced glycation products (AGEs) and decreased pancreatic beta cells. Shahab et al. [48] reported that WDL has anti-glycating and anti-oxidation properties. A recent study has demonstrated that WDL has apparent lipid-lowering impacts and its underlying mechanism is mediated by peroxisome proliferator-activated receptor-α (PPARα)/lipoprotein lipase (LPL) and low-density lipoprotein receptor (LDLR) up-regulated expression and AMPK activation. Furthermore, WDL has been shown to cure hepatic steatosis. By

directly scavenging reactive oxygen species (ROS) and raising the activity of several antioxidant enzymes, WDL shielded the liver from ROS attack [49]. It has been recently reported that regulation of Bcl-2 family protein expression by WDL-gold nanoparticles might be used as a promising approach to reduce the levels of lipid peroxidation, improve antioxidant, increase insulin secretion and activate the PI3K/Akt pathway in RIN-5F cells/rat [50] (Fig. 2). Kumar et al. [51] has studied the hypoglycemic effect of WDL using α-amylase and α-glucosidase properties [51]. It was noted that the inhibitory effect on α-glucosidase of WDL was as same as to the standard drug acarbose, i.e., 80.65% and α-amylase activity showed much higher potential than acarbose, which is 93.83% while acarbose showed only 42.23%. Further, molecular docking of WDL with glibenclamide has also been studied. WDL showed anti-diabetic activity in the zebrafish model [52].

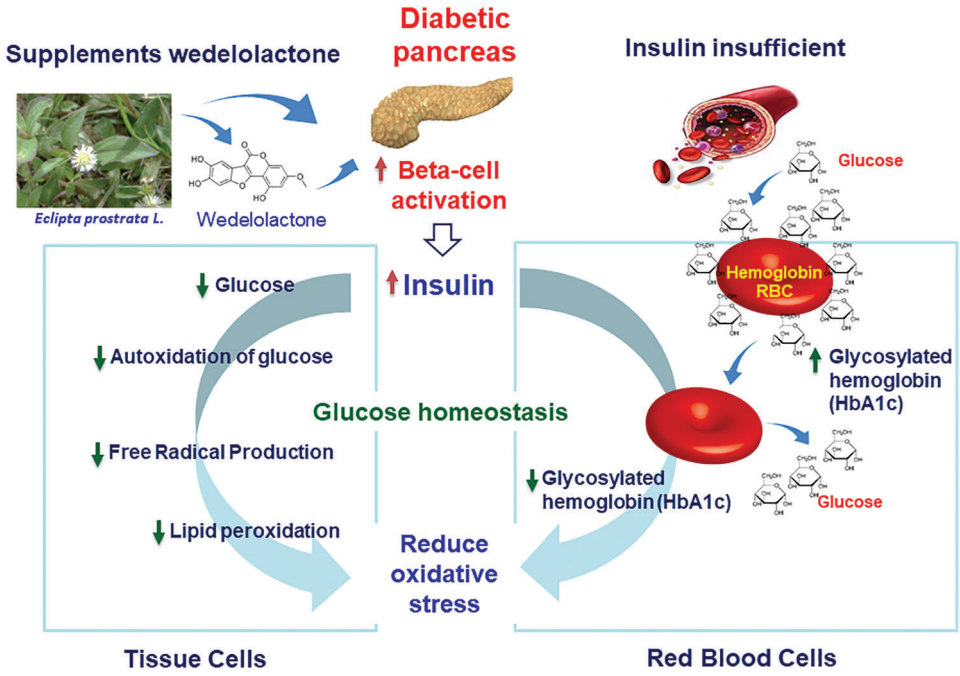

Figure 2: Scheme indicates the mechanism of oxidative stress reduction of WDL. Diabetes causes destruction of beta-cells, results in decreased insulin level that leads to significant increase in blood glucose level. Increased glucose binds to protein molecules and as result increases glycosylated haemoglobin (HbA1c). WDL treatment protects beta-cells from diabetic condition free radicals *via* its antioxidant activity thereby improve insulin levels and decrease glucose in blood leading to reduced HbA1c. WDL treatment significantly decreases the glucose auto-oxidation, free radical levels, lipid peroxidation, and improves antioxidants

4.5 Anti-Inflammatory

Many studies showed that WDL and its active constituents possessed anti-inflammatory properties. WDL, a natural compound has been shown to inhibit the activation of nuclear factor-kappa B (NF-κB) pathway. It possesses anti-inflammatory activity to inhibit cytokines including IL-1b and IKK in disease condition [53]. The results showed that WDL from *E. alba* could be a novel drug for the therapy of kidney damage caused by the inflammation response of human renal mesangial cells through influencing the NF-κB signaling pathway [54]. Furthermore, the treatment of WDL (30 μg/ml) significantly decreased phosphorylation of p47phox, superoxide generation, and nicotinamide adenine dinucleotide phosphate oxidase [42]. A study by Yuan et al. [55] found that WDL (10μM) treatment inhibited the

inducible nitric oxides synthase (iNOS) and cyclooxygenase 2(Cox2) protein expression in lipopolysaccharide-stimulated cells.

4.6 Neurotoxicity

Some individuals, especially elderly people, have a high risk for degenerative nerve diseases such as Parkinson's and Alzheimer diseases. Recent investigations revealed that WDL could protect motor neurons from Al-caused toxicity by enhancing antioxidant status, brain-derived neurotrophic factor, and prevent excitotoxicity of glutamate. In brain, excitotoxicity can cause neuron damage by the over-activations and excessive stimulation of receptors for the neurotransmitter glutamate, NMDA receptor for N-methyl-D-aspartic acid (NMDA), and AMPA receptor for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Moreover, WDL inhibits the activation of caspase-3 and reduces inflammatory cytokines [56]. In addition, WDL therapy reduced lactate dehydrogenase (LDH), caspase-3 and m-calpain operations. The reports highly supported the usefulness of both compounds in preventing quinolinic acid-induced glutamate excitotoxicity. After WDL treatment, vascular endothelial growth factor (VEGF), insulin-like growth factor type 1 (IGF-1) and N-acetylasparatate (NAA) levels were improved in the brain [57].

4.7 Cardiovascular

Cardiovascular diseases (CVD) are heart and blood vessel disorders such as coronary heart disease, hypertensive heart diseases, stroke, rheumatic heart disease and other conditions. Four of five deaths from CVD are caused by heart attacks and strokes. WDL has exhibited cardiovascular protective effects by dyslipidemia, such as in the reduced triglycerides (TG), very low-density lipoprotein cholesterol (VLDL-C), total cholesterol (TC), upregulation of PPAR-α and activation of AMPK [49]. Another report shows that WDL may have therapeutic effect by activating AMPK and significantly decreasing protein expression TGFβ/Raf-MAPK [58]. The administration of WDL suppressed Akt and neointima to and inhibited cyclin D1 and cyclin-dependent kinase inhibitor p^{21} in balloon-injured common carotid arteries (CCAs) when compared with untreated CCAs. Based on these results, it can be stated that WDL exhibits potential therapeutic efficacy in treating cardiovascular ailments in rats [44].

4.8 Osteoporosis

Osteoporosis, a chronic metabolic bone disease, is a major health problem. Studies have found that WDL exerted a promising beneficial effect on osteoclast differentiation and osteoblastogenesis protein markers such as osteocalcin, runx2 and osteorix, which can encumber osteoclastic bone resorption. It has also been shown that WDL inhibits migration of MV3 cells. WDL caused the development of Bax pro-apoptotic protein but inhibited the development of apoptotic Bcl-2 protein. WDL inhibited cycline D expression while significantly improving p21 protein expression. WDL has been shown to inhibit the activation of Akt but cause activation of AMPK [59]. Studies have also demonstrated that WDL can efficiently protect mesenchymal stem cells against hydroxyl radical-induced oxidative damage. This protective effect may provide application of WDL in mesenchymal stem cells transplantation (osteoporosis); radical scavengers *via* an electron transfer- radical adduct formation reaction RAF pathway [29]. It has been evaluated that WDL semaphoring 4D (SEMA4D) inhibits the formation of SEMA4D/PLEXIN-B1. The cell culture of bone marrow stromal cells with RAW264.7 cell, addition, WDL at the dose of tartrate-resistant acid phosphatase properties. The study demonstrated that WDL reduced the progression of osteoclastogenesis but stimulated osteoblastogenesis [60]. Moreover, the gene expression bone morphogenetic protein-2 and phosphorylation levels of SMAD-1/5/8 were significantly decreased after WDL treatment [61].

4.9 Hepatoprotective

Hepatitis is an inflammation of the liver that can cause a range of health problems. The liver is one of the body's main organs and plays a basic role in regulating various functions, including metabolism, secretion, storage, and detoxification of endogenous and exogenous substances. WDL improved hepatic lipid metabolism and improves hepatic steatosis mediated by AMPK activation [49]. Luo et al. [62] showed that WDL could suppress NF-κB activity, a significant transcription factor for inflammatory cytokines by restricting IκB and p65 phosphorylation. The authors of the studies concluded that WDL shows hepatoprotective activity by inhibiting topoisomerase activity [63].

Table 1: Biological activities of WDL

Diseases	Function pharmacological actions	Models	References
Diabetic	↓ Immune-cell infiltration.	Zebrafish	[52]
	↑ Oxidative stress markers and ↓ Glycated protein and glucose levels.	Rats	[48]
	Bcl-2 family protein expression by WDL-AuNPs might be used as a promising approach to reduce the levels of lipid peroxidation. ↑Antioxidant and insulin secretion.	RIN-5F cell line/rats	[50]
Cancer	Inhibited MV3 cell proliferation, invasion, and migration. Inhibited Bcl-2, cyclin D expression, and Akt activation and induced AMPK activation while ↑ p21 protein expression.	MV3 cells	[44]
	↓ Percentage of ALDH+BCSCs and $cd44^{+}/cd24^{-}$ low population.	MDA-MB-231 cells	[45]
	↑The lysosomal level of copper, and it the main prooxidative induced by WDL taking place inside the nucleus.	MDA-MB-231 and T47D cells	[64]
Osteoporosis	↑Osteoblastogenesis genes expression marker such as RUNX2, osteorix and osteocalcin.	BMS cells	[59]
	Enhanced osteoblastogenesis through promoting sema7A production and sema7A-PlexinC1-beta1 complex formation.	BMS and RAW264.7 cells	[60]
	Sema3A/neuropilin-1 (NRP1) pathway-mediated β-catenin activation and NF-κB pathway inhibition	Dental pulp stem cells	[65]
	Enhanced osteoblastogenesis through induction of ERK- and JNK-mediated expression of BMP2 and phosphorylation SAMAD1/5/8 pathways.		[61]
Neuroprotective	Inhibited casp-3activated and decreased inflammatory and increased motor learning abilities and co-ordination motor.	Wistar rats	[57]
	↓Protein expression of TNF-alpha, IL-6 and IL-beta. ↓Protein expression NF-κB by immunohistochemicals.	Wistar rats	[56]

(Continued)

Table 1 (continued).

Diseases	Function pharmacological actions	Models	References
Anti-Inflammation	↓Phosphorylation of p47phox, NADPH, and Supreoxide generation	Mice	[42]
	Inhibited NLRP3 inflammasome activation and phosphorylation casp-1 to ↓ IL-1β released.	Mouse	[66]
	Inhibit the abnormal proliferation of HRMCs *via* regulating the properties of NF-κB signaling pathway.	Human renal mesangial cells	[54]
	↓the levels of cytokines IL-6, MCP-1, TNF-alpha, and TGF-beta 1. Additionally, the activation and phosphorylation of I kappa Ka, I kappa Ba and NF-kappa B p65 was inhibited by WDL. ↓NF-kappa B p65 phosphorylation.	MPC-5 cells	[67]
Hepatoprotective	Suppressed the activity of NF-κB, a critical transcriptional factor of inflammatory cytokines by limiting the phosphorylation of IκB and p65.	Mice	[62]
	↓the liver index, AST, ALT levels and liver tissue xanthine oxidase, malondialdehyde, iNOSand TNF-alpha.↑ The liver tissue SOD,GPx, IL-4 and IL-10.	D-galactosamine induced liver injury in mice	[68]
Androgenetic Alopecia	Enhanced hair follicles and stimulated hair growth	Mice	[30] [69]

4.10 Promotion of Hair Growth

Androgenetic alopecia (AGA) is commonly related with aging type of hair loss symptoms in men [70]. AGA affects up to 80% men and 50% women in their lifetime [71]. It is the result of the effects of dehydrotestosterone (DHT) converted from testosterone by enzymatic functions of 5-alpha reductase. High dose of DHT affect androgen-sensitive hair follicles and cause progressing hair loss [72]. Another type of hair loss, *Alopecia areata* is caused by autoimmune disorder in hair follicles that results in hair loss resulting in spot baldness [73]. Currently, inhibitors for a type 25-α reductase, such as finasteride, dutasteride and minoxidil, are main drugs in treating AGA and promoting hair regrowth [74,75]. In order to reduce side effects, AGA hair loss has been treated with natural products even in a traditional way without using drugs such as finasteride and dutasteride. Formulation of herbal extracts and natural compounds is mostly applied to reduce progression of hair loss [75]. *E. alba* showed hair growth promotion effects in traditional medicine in Korea and China. Evidential examples have been applied to prevent hair loss using *E. alba* extracts which increased the hair follicles and improved hair growth in mice [30,69] (Fig. 3 and Tab. 1).

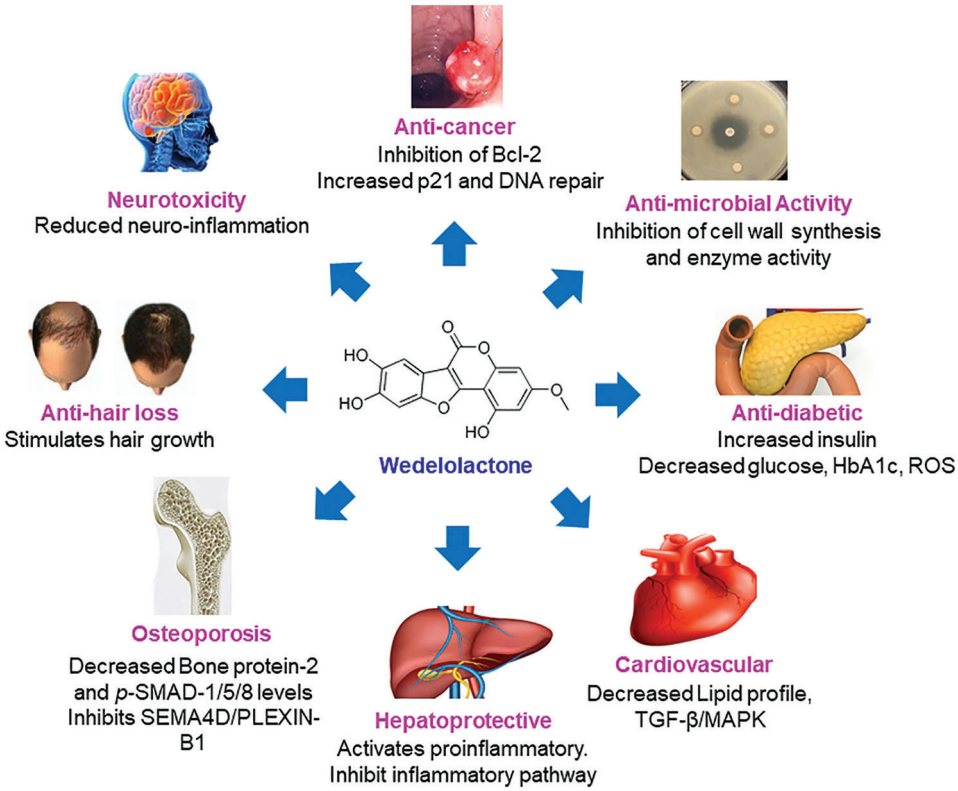

Figure 3: Pharmacological mechanisms of WDL

5 Conclusion

Natural fresh plant sources of high phytochemicals and antioxidants have the potential to be useful supplements in the discipline of integrative medicine. This review presents comprehensive information derived from recently published scientific works that have documented the biological activity of WDL in therapeutic applications. The antibacterial, analgesic, antioxidant, cytotoxic, antidiabetic, anti-inflammatory, neuroprotective, cardioprotective and hepatoprotective activities of WDL and the mechanisms of action have also been discussed in this review.

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