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Wedelolactone: A Natural Anti-inflammatory Coumarin Derivative

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Abstract
Medicinal herbs have been extensively studied and utilized for centuries in many developing countries as an effective approach to treat various diseases and health conditions. Wedelolactone (WDL), a key bioactive compound found in Eclipta prostrata (also known as Eclipta alba), has shown promising potential for promoting human health, particularly in combating chronic illnesses. However, a thorough investigation into the full range of WDL's pharmacological properties across different conditions has not yet been conducted. This review aims to highlight recent therapeutic research regarding the biological and functional activities of WDL in relation to major chronic diseases, such as cardiovascular disorders, cancer, diabetes, liver disease, Alzheimer's disease, and androgenetic alopecia. We compiled relevant experimental data on WDL from trusted scientific databases, including PubMed, Web of Science, ScienceDirect, and Google Scholar. In conclusion, WDL stands out as a potent antioxidant and a selective modulator of key proteins associated with chronic diseases. Given its therapeutic potential, further exploration of WDL's pharmacological effects is strongly recommended.

Keywords: Wedelolactone, Eclipta Prostrata, Anti-Inflammatory, Coumarin.

Introduction:

Wedelolactone is obtained from leaves of Eclipta prostrata linn belonging to family Asteraceae. Eclipta prostrata (L.), commonly known as E. prostrata, is a traditional medicinal herb used for centuries in many regions worldwide. It is known for its impressive healing benefits, including treating liver damage from alcohol consumption and jaundice, as well as addressing inflammatory issues[1] .Eclipta prostrata also known as *Bhringraj, Bhumiraj, Aali jhar,* and *Nash jhar* in Nepali language.[2,3].
As per Ayurvedic Pharmacopoeia of India this plant is considered as hepatoprotective. The full taxonomic hierchy is given below:-

Scientific Classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Eclipta L.
Species: Eclipta prostrata
Vernacular Names
English: Bhringaraj
Sanskrit: Bhringaraj
Hindi: Bhangara, Bhangaraiya

Bengali: Kesuriya, Kesari
Tamil: Karisalai
Marathi: Maka
Telugu: Guntagalagara

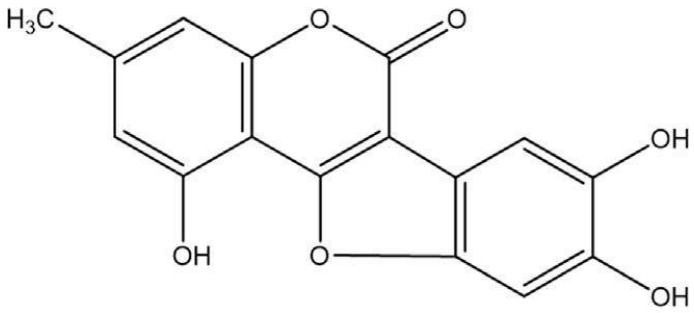

Fig1-Wedelolactone

Chemical name: 7-methoxy 5,11,12-trihydroxy-coumestan
Chemical formula: C16H10O7
Molar mass: 314.249 g/Mol
Boiling point: 498.4°C at 760 mmHg
Density: 1.655 g/cm3
Pubmed CID: 5281813

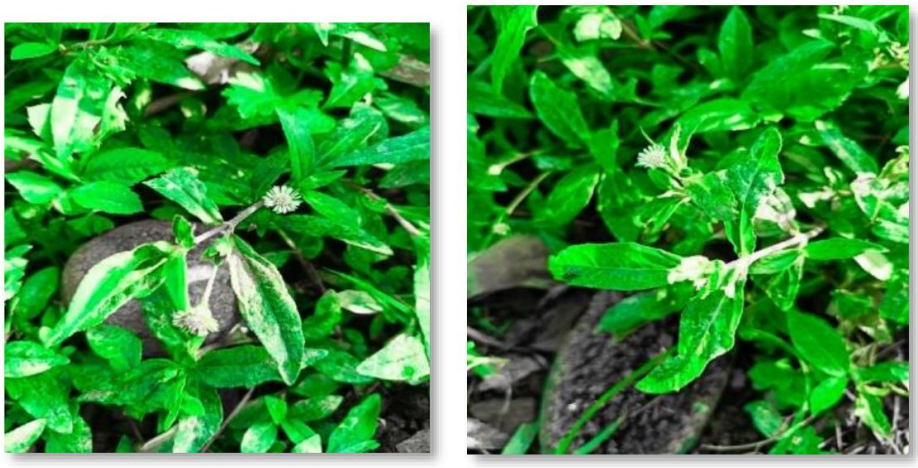

Fig. 2- Eclipta prostrata

Methos of Extraction:

1. Method 1

The leaf powder was soaked in 95% methanol for 72 hours, with periodic shaking at ambient temperature. Afterward, the extract was transferred to a conical flask, filtered using Whatman No.1 filter paper, and the resulting filtrate was evaporated under reduced pressure until dry. The extract yielded approximately 8.7% w/w.[4]

2. Method 2

A 100-gram sample of shade-dried Eclipta prostrata leaves was ground into powder using a pulverizer. This powdered material was placed in a thimble and positioned in the Soxhlet extractor containing 500 mL of solvent. Approximately 1.5 liters of absolute ethanol was used to extract the plant's crude metabolites through 16 cycles of reflux. The solvent containing the extracted metabolites was then evaporated and condensed using a rotary evaporator.[5]

3. Method 3

The whole plant of Eclipta alba was chopped into small pieces using a knife. Two separate 2000 mL conical flasks were each filled with 250 g of dried plant material, and 1000 mL of methanol and 1000 mL of petroleum ether were added to the respective flasks. The mixtures were left for 72 hours in airtight containers at a temperature of 25 to 30°C. Afterward, the extracts were filtered using standard filter paper, and the filtrate was collected in a 1000 mL beaker. The filtrate was then concentrated using a rotary evaporator at 40 to 45°C under standard conditions. The extraction yielded approximately 1.16% w/w, and the final extract was stored in glass vials in airtight conditions at room temperature, properly labeled.[6]

4. Method 4

The dried and powdered plant material (including leaves, roots, stems, seeds, and aerial parts) was subjected to extraction using chloroform in a Soxhlet apparatus. After the solvent was evaporated under reduced pressure, a semi-solid residue was obtained, with a yield of 16.7%. The extract tested positive for the presence of alkaloids, volatile oils, and saponins. Different doses of the extract (50, 100, and 200 mg/kg) were then suspended in a 2% aqueous Tween 80 solution.[7]

5. Method 5

After harvesting, the plant parts were thoroughly washed with water and air-dried at temperatures between 35-50°C. Once dried, the plant material was ground into a coarse powder. Approximately 600 g of this powder was placed in an amber glass container and soaked in 3 liters of 95% ethanol at room temperature for several days. The mixture was then filtered through Whatman No. 1 filter paper. The resulting filtrate was evaporated using a rotary evaporator under reduced temperature and pressure. Following this, the crude extract of E. alba (15.0 g) underwent solvent-solvent partitioning using the modified Kupchan method, yielding n-hexane (3.27 g), chloroform (2.05 g), and an aqueous fraction (2.13 g). All these fractions were subjected to phytochemical and pharmacological testing.[8]

6. Soxhlet Extraction method-

The Soxhlet apparatus was employed to extract Wedelolactone (WDL) from the aerial parts of *E. alba*. The plant material, which had been dried in the shade and ground into a powder, was subjected to extraction using different solvents, including water, hexane, absolute methanol, and absolute ethanol, at 50°C for 36 hours. After extraction, the material was filtered, concentrated using a rotary evaporator, and the resulting residue was stored at 4°C until needed for further analysis. Additionally, the extracted sample was analyzed for various phytochemical components.[9]

7. Ultrasonic Assisted extraction

In this technique, an ultrasonic probe was used in an sonochemical reactor to extract Wedelolactone (WDL) from E. prostrata. To optimize the extraction process, response surface methodology and central composite design were employed. Key parameters for optimization included solvent type, temperature, extraction time, solvent-to-material ratio, ultrasonic power, and extraction duration. During the process, the powdered plant material (screened through a #80-100 standard filter) was soaked in the solvent for about one hour in a vessel. Ultrasonication was then applied using a 50% duty cycle, with the probe immersed 1.0 cm into the solvent, alternating between 5 seconds ON and 5 seconds OFF. Savita et al. utilized this approach to extract WDL from E. alba using a 1:5 methanol-to-herb powder ratio, yielding 0.36% WDL.

8. Mceration

In this procedure, air-dried and coarsely powdered E. alba whole plant was macerated in methanol for 24 hours, followed by percolation in a vessel until a colorless liquid was collected [19]. The resulting percolate was concentrated using a vacuum rotary evaporator at 40°C, producing a sticky green mass. The isolation of Wedelolactone (WDL) was carried out through column chromatography, employing 60–120

mesh silica gel and toluene as the eluting solvent. The isolated compound was further purified using preparative high-performance thin-layer chromatography, yielding 0.38%. The purified WDL was then characterized and confirmed through various spectroscopic methods.[10]

9. Supercritical Exraction

Patil et al. used the supercritical CO2 extraction method to isolate Wedelolactone (WDL) from W. calendulacea. In this approach, the sample powder was loaded into an extraction column, which was then placed in a column oven. A chiller unit was used to circulate CO2 and maintain the desired pressure. Methanol was introduced into the system using a solvent pump. The entire setup was temperature-controlled, and the extraction process began when six-port valves were opened to allow supercritical CO2 to flow through the column. The extraction was performed in two phases: a static first cycle to ensure complete sample contact, followed by a dynamic second cycle where CO2 flowed steadily through the sample. The collected extract was analyzed to confirm the presence of WDL. They observed that increasing both the pressure (from 25.0 to 35 MPa) and temperature (from 40 to 80°C) resulted in a decrease in WDL concentration, whereas increasing the extraction time from 30 to 90 minutes led to higher WDL levels.[11]

10. Microwave Assisted Extraction-

Dang et al. employed a reflux system with a round-bottom flask to extract Wedelolactone (WDL) from E. alba. In their method, 90% ethanol was used as the extraction solvent, and the sample was subjected to 200W microwave radiation for 30 minutes. The mixture was then filtered, and the extraction process was repeated three times with the same sample. Following this, the extract was concentrated using a rotary vacuum evaporator at 50°C. The dried extract was dissolved in hot water, filtered, and then extracted three times with ethyl acetate. The ethyl acetate extracts were dehydrated using anhydrous sodium sulfate, and approximately 80%–90% of the solvent was evaporated under vacuum at 50°C. To purify the WDL, a silica gel column chromatography was performed using a mobile phase consisting of dichloromethane, methanol, and a small amount of acetic acid. The final purification was carried out through dilution crystallization, optimized using single factor analysis and response surface methodology. This process resulted in a 77.66% yield and a 99.46% purity of WDL.[12]

11. Ultra-High Pressure-Assisted Extraction

In this technique, high-speed counter-current chromatography (HSCCC) was combined with ultra-high-pressure extraction (UHPE) to isolate and purify isodemethyl-WDL and Wedelolactone (WDL) from E. alba. The crude powdered herb was sieved through a 60–80 mesh, then placed into a polyethylene bag with the extraction solvent, sealed, and subjected to extraction in an ultra-high-pressure vessel. After extraction, the mixture was centrifuged at 6000 rpm for 5 minutes and filtered through a 0.45-micron membrane. The WDL content was quantified using high-performance liquid chromatography (HPLC). For the HSCCC separation, a two-phase solvent system composed of petroleum ether, ethyl acetate, methanol, and water in a 3:7:5:5 ratio was used. This method successfully yielded 23.5 mg of WDL, 6.8 mg of isodemethyl-WDL, and 5.5 mg of luteolin from 300 mg of crude extract, all with greater than 95% purity in a single-step separation. Various UHPE parameters, including extraction time, solvent, pressure, and solid-liquid ratio, were optimized using an orthogonal array. The optimal conditions for achieving the highest yield were found to be 80% aqueous methanol, 3 minutes of extraction, a solid-liquid ratio of 1:20, and a pressure of 200 MPa.[13]

12. Aqueous Two-Phase System (ATPS) Extraction Method

In this approach, an aqueous two-phase system (ATPS) was created using polyethylene glycol (PEG) of varying molecular weights and sodium citrate salt . A specific amount of dried E. alba powder was placed in a conical flask at a predetermined pH. The mixture was then agitated for 2 hours at 30 ± 2°C and 600 rpm using a magnetic stirrer. Following agitation, the mixture was centrifuged at 8000 g for 10 minutes,

and the resulting phases were separated using a separating funnel. The extracted components were analyzed using high-performance liquid chromatography. It was observed that as the concentration of sodium citrate, PEG molecular weight, PEG concentration, and pH increased (from 14% to 16%, 4000 to 6000, 12% to 18% w/v, and pH 5 to 7, respectively), the yield increased. However, further increases in these parameters led to a reduction in yield [23]. The optimal conditions for the ATPS system were found to be PEG 6000, with an 18% (w/v) PEG concentration, 17.96% (w/v) sodium citrate, and a pH of 7, resulting in an extraction yield of 6.73 mg/g.[14]

Anti-Inflammatory Activity-

Inhibition of Egg Albumin Denaturation:

The anti-inflammatory activity of E. alba extract was assessed using the egg albumin denaturation assay (Ahamed et al., 2021). In brief, 1.0 ml of 5% egg albumin solution and 2.8 ml phosphate buffer (pH 6.4 ± 0.2) were combined in sterile test tubes. Aspirin (0.1 mg) and Tween-80 served as positive and negative controls, while the test sample of E. alba (500 µg/ml in Tween-80) was used as the test group. The mixtures were heated at 57°C for 20 minutes, then cooled and filtered. Absorbance was measured at 660 nm using a UV-visible spectrophotometer for each concentration (125, 250, and 500 µg/ml). Anti-inflammatory activity was calculated based on the percentage inhibition of protein denaturation.

$$\% \text{ Inhibition of protein denaturation} = \frac{A_{control} - A_{test}}{A_{control}} \times 100\%$$

Here, A= Absorbance for respective group.

Membrane Stabilization Activity Assay:

In this assay, 1.0 ml of phosphate buffer (pH 7.4), 2.0 ml of hyposaline, and 0.5 ml of human RBC suspension were added to sterile tubes. Aspirin (0.1 mg) was used for the standard group, distilled water (1.0 ml) served as the control, and varying concentrations of the plant sample (125, 250, and 500 µg/ml) were added to the test groups. The tubes were incubated at 37°C for 30 minutes, followed by centrifugation at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 560 nm using a UV-visible spectrophotometer. Membrane stabilization activity was calculated based on the absorbance readings.

$$\% \text{ Inhibition of Hemolysis} = \frac{A_{control} - A_{test}}{A_{control}} \times 100\%$$

Here, A= Absorbance for respective group. [8]

Anti-Inflammatory Potential of Methanolic Extract of E. prostrata Leaves:

The anti-inflammatory activity of the methanolic extract of E. prostrata leaves was evaluated using carrageenan-induced rat paw oedema and egg white-induced hind paw oedema models.

In the carrageenan-induced paw oedema test, the extract at 100 and 200 mg/kg doses exhibited 34.02% and 38.80% inhibition, respectively, compared to indomethacin, which showed 48.47% inhibition. In the egg white-induced oedema test, the extract at the same doses showed 35.05% and 38.23% inhibition, while the standard drug, cyproheptadine (8 mg/kg), produced 56.09% inhibition.

The results suggest that the anti-inflammatory effect of the methanolic extract of E. prostrata leaves is comparable to the standard drugs used in the study. Both carrageenan- and egg white-induced oedema models are frequently employed to evaluate anti-inflammatory properties of natural products.

The anti-inflammatory effect is likely due to the inhibition of various inflammatory mediators, such as histamine, serotonin, kinins, prostaglandins, and cytokines, which are known to play key roles in

inflammation. The extract's effect during both phases of oedema development indicates inhibition of mediators like histamine and serotonin, which are involved in the early stages of inflammation. This suggests that E. prostrata may exert its anti-inflammatory action by targeting the release of these mediators and other inflammatory substances.[4]

Wedelolactone extracted from leaves of Eclipta prostrata by using methanol as a solvent using Soxhlet Extraction method. Wedelolactone inhibits Lipopolysaccharide(LPS) induced proinflammatory cytokines such as Tumour Necrotic Factor-α(TNF-α), Interleukin-6,Interleukin-8 and no activity on Interleukin-10 also reduce generation of reactive oxygen species(ROS) which are responsible for inflammatory response.

HPLC of Wedelolactone-

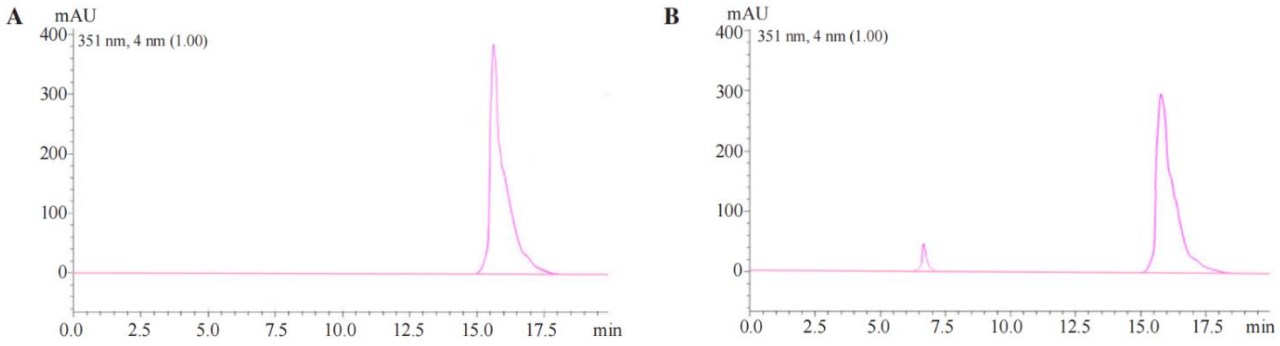

Fig 3-HPLC analysis of wedelolactone isolated from Eclipta prostrata (L.) L. (A) Standard wedelolactone; (B) Sample wedelolactone.

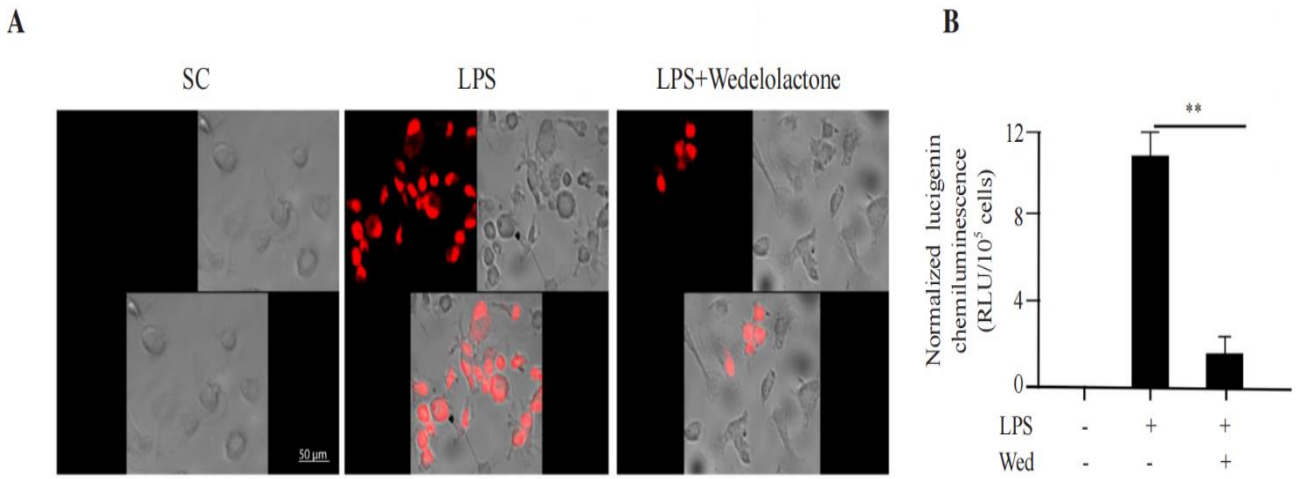

Fig 4-Effect of wedelolactone on the formation of reactive oxygen species and NADPH oxidase activity in LPS-induced Raw 264.7 cells. (A) The fluorescence intensity of the cells (scale bar: 50 μm). (B) NADPH in the cells. Data are presented as mean ± SD of three independent experiments and analyzed by one-way ANOVA analysis. **P<0.01 compared with the LPS-stimulated group. SC: solvent control.[15]

Wedelolactone also has anti inflammatory activity against Zymosen induced inflammation.Zymosen is a ß-glucan component which is isolated from Saccharomyces cereviceae which is used to induce inflammation both in in-vivo and in-vitro.[16]

Other Activities of Wedelolactone-

Antioxident-

Free radicals are highly reactive molecules with an unpaired electron in their outermost orbitals, which makes them seek out electrons from other substances to stabilize themselves. Excessive generation of free radicals, including reactive oxygen and nitrogen species (RONS), has been implicated in the onset of various chronic diseases. Studies have shown that Wedelolactone (WDL) extracted from E. alba possesses significant antioxidant activity, enabling it to effectively neutralize nitric oxide radicals and superoxide anions.[17] Mechanistic studies revealed that the primary pathway involves single electron transfer, while Fe2+ chelation acts as a secondary mechanism. Both pathways are attributed to the catechol group in Wedelolactone (WDL), rather than to the cumestane structure.[18]

Antimicrobial-

In recent years, phytochemicals with antimicrobial properties have attracted significant interest for pharmaceutical use. Among bacterial species, Staphylococcus epidermidis, Staphylococcus aureus, and Salmonella typhimurium are particularly susceptible to the antimicrobial effects of Wedelolactone (WDL), while Shigella flexneri exhibited the highest resistance. These results suggest that WDL holds potential as an effective antimicrobial agent.[19]

Antidiabetic Activity-

Diabetes is primarily marked by elevated blood sugar levels. Over the last ten years, research has demonstrated that Wedelolactone (WDL) possesses strong antioxidant and antiglycation properties. Notably, reduced levels of glycosylated hemoglobin (HbA1c) and glycated serum proteins suggest a lower formation of advanced glycation end products (AGEs) and a decrease in the damage to pancreatic beta cells. Wedelolactone (WDL) exhibits both anti-glycating and antioxidant activities. Recent research has highlighted its significant lipid-lowering effects, with the underlying mechanism involving the upregulation of peroxisome proliferator-activated receptor-α (PPARα), lipoprotein lipase (LPL), and low-density lipoprotein receptor (LDLR) expression, along with activation of AMP-activated protein kinase (AMPK). Additionally, WDL has been shown to be effective in treating hepatic steatosis. [20]

The hypoglycemic effects of Wedelolactone (WDL) were investigated through its activity on α-amylase and α-glucosidase enzymes. It was observed that WDL inhibited α-glucosidase to the same extent as the standard drug acarbose, with an inhibition rate of 80.65%. However, WDL demonstrated a significantly stronger inhibition of α-amylase, showing 93.83% activity, compared to acarbose, which only exhibited 42.23% inhibition.[21]

Cardiovascular effect-

Cardiovascular diseases (CVD) encompass a range of heart and blood vessel disorders, including coronary heart disease, hypertensive heart disease, stroke, and rheumatic heart disease, among others. Heart attacks and strokes account for four out of five deaths related to CVD. Wedelolactone (WDL) has demonstrated protective effects on cardiovascular health by improving dyslipidemia, such as lowering triglycerides (TG), very low-density lipoprotein cholesterol (VLDL-C), and total cholesterol (TC). This is achieved through the upregulation of PPAR-α and activation of AMPK.[22]

Osteoporosis-

Osteoporosis, a long-term metabolic bone disorder, represents a significant public health issue. Research has indicated that Wedelolactone (WDL) offers potential benefits in regulating osteoclast differentiation and promoting osteoblastogenesis, as evidenced by the modulation of key protein markers like osteocalcin, Runx2, and osterix, which help inhibit osteoclastic bone resorption. Additionally, WDL has been found to hinder the migration of MV3 cells. It promotes the expression of the pro-apoptotic protein Bax while

suppressing the anti-apoptotic Bcl-2 protein. WDL also reduces cyclin D expression and enhances p21 protein levels. Furthermore, WDL has been shown to inhibit Akt activation and stimulate AMPK activity.[23]

Research has shown that Wedelolactone (WDL) effectively protects mesenchymal stem cells from oxidative damage induced by hydroxyl radicals. This protective effect may have potential applications in mesenchymal stem cell transplantation, particularly for conditions like osteoporosis, acting as a radical scavenger through an electron transfer-radical adduct formation (RAF) pathway. Furthermore, studies have indicated that WDL inhibits the formation of SEMA4D/PLEXIN-B1 complexes by modulating semaphorin 4D (SEMA4D). In co-cultures of bone marrow stromal cells and RAW264.7 cells, WDL at a specified dose was shown to affect tartrate-resistant acid phosphatase activity. The findings suggest that WDL can slow down osteoclastogenesis while promoting osteoblastogenesis.[24]

Hepatoprotective-

Hepatitis is a condition characterized by inflammation of the liver, leading to a variety of health complications. As one of the body's key organs, the liver is essential for regulating processes such as metabolism, secretion, storage, and detoxification of both internal and external substances. Wedelolactone (WDL) has been shown to enhance hepatic lipid metabolism and alleviate hepatic steatosis, primarily through the activation of AMPK.[25]

The studies demonstrated that Wedelolactone (WDL) could inhibit NF-κB activity, a key transcription factor involved in regulating inflammatory cytokines, by limiting the phosphorylation of IκB and p65. The researchers concluded that WDL exhibits hepatoprotective properties, partly through the suppression of topoisomerase activity.[26,27]

Andogenic Alopesia-

Androgenetic alopecia (AGA) is a common form of hair loss associated with aging in men [28].

It affects up to 80% of men and 50% of women over the course of their lives.[29]

Androgenetic alopecia is caused by the effects of dehydrotestosterone (DHT), which is produced from testosterone through the enzymatic action of 5-alpha reductase. Elevated levels of DHT target androgen-sensitive hair follicles, leading to progressive hair loss.[30]

Another type of hair loss, Alopecia areata, is an autoimmune condition where the immune system mistakenly attacks hair follicles, leading to hair loss and the formation of bald patches.[31]

At present, inhibitors of 5-alpha reductase, such as finasteride, dutasteride, and minoxidil, are the primary medications used to treat androgenetic alopecia (AGA) and promote hair regrowth.[32,33]

To minimize side effects, natural products have been used to treat androgenetic alopecia (AGA) in a more traditional manner, without relying on drugs like finasteride and dutasteride. The use of herbal extracts and natural compounds is often employed to help slow down the progression of hair loss.[34]

E. alba has been recognized for its hair growth-promoting effects in traditional medicine in both Korea and China. There is documented evidence showing that E. alba extracts can help prevent hair loss by stimulating hair follicle activity and enhancing hair growth in mice.[35]

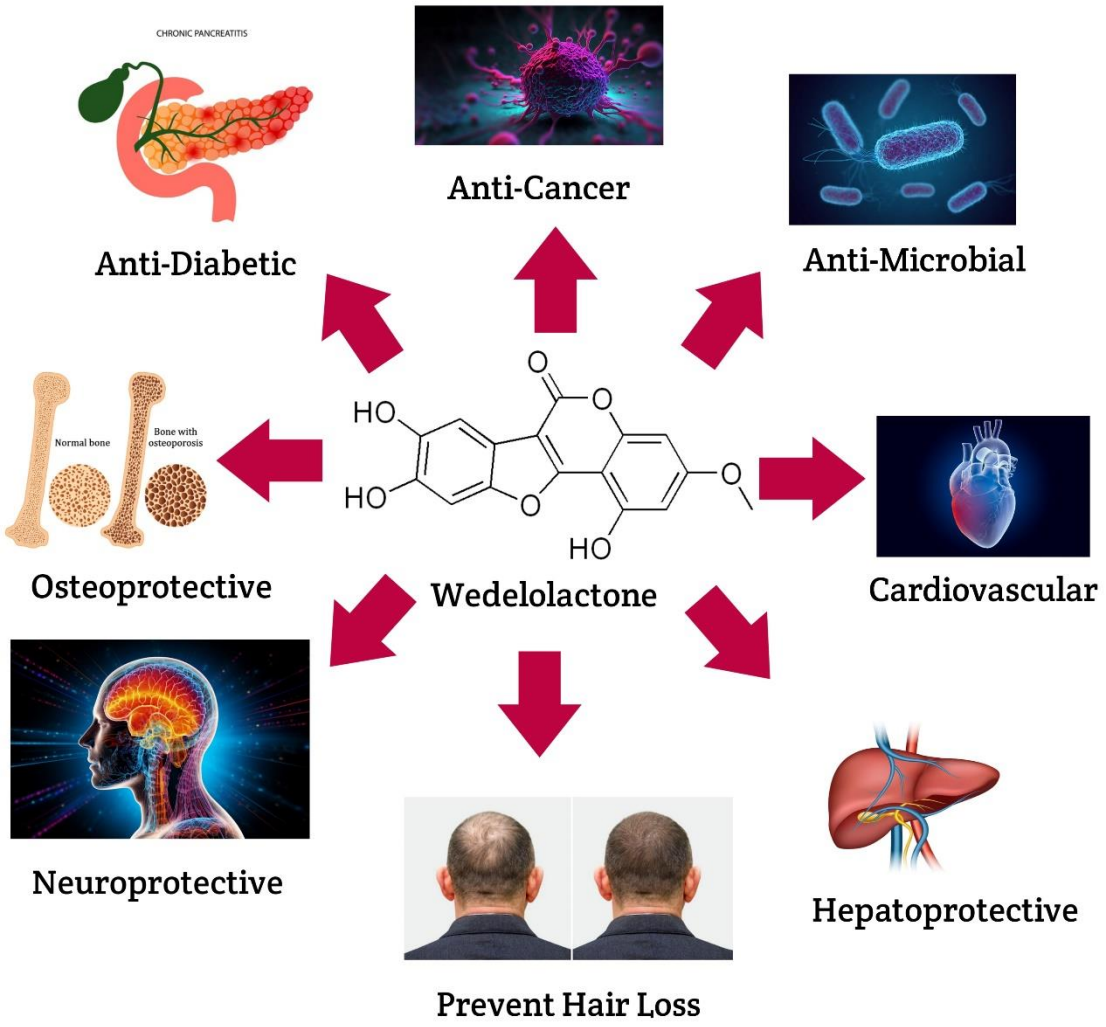

Fig. 4-Uses of Wedelolactone

Mechanism of action of Wedelolactone-

Inflammation is a vital response of the host to tissue injury or foreign invaders, aimed at restoring tissue integrity and function. This process is driven by the activation of immune cells, particularly macrophages, through pro-inflammatory cytokines, which enhance the inflammatory response. Macrophages, along with neutrophils and dendritic cells, play a central role in the innate immune defense. When activated, macrophages release several key inflammatory mediators such as nitric oxide (NO), inducible nitric oxide synthase (iNOS), prostaglandin E2 (PGE2), cyclooxygenase-2 (COX-2), and the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α). Lipopolysaccharide (LPS), a component of Gram-negative bacterial cell walls, triggers macrophages to produce these mediators, simulating an in vivo inflammatory response. LPS initiates a cascade of signaling events that activate the NF-κB and mitogen-activated protein kinase (MAPK) pathways, both of which are well-established models for studying innate immunity. NF-κB plays a critical role in regulating immune and inflammatory responses by controlling the expression of pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes like COX-2 and iNOS. TNF-α activates NF-κB by promoting the phosphorylation, ubiquitination, and degradation of its inhibitor IκBα. Furthermore, MAPKs, including ERK1/2, JNK, and p38, are involved in various cellular processes such as proliferation, differentiation, survival, and apoptosis. MAPKs can be

activated by Toll-like receptor 4 (TLR4), leading to NF-κB nuclear translocation and triggering pro-inflammatory responses. Once activated, NF-κB moves into the nucleus, where it regulates the expression of genes associated with inflammation and cell survival.

Effects of WEL on iNOS and COX-2 Protein Expression in LPS-Stimulated Cells:
To determine whether the inhibition of WEL on NO and PGE2 production was linked to the downregulation of iNOS and COX-2, we conducted further experiments. Cells were pretreated with varying concentrations of WEL for 12 hours, followed by LPS (1 μg/ml) treatment for an additional 20 hours. LPS stimulation significantly increased the protein levels of iNOS and COX-2, while WEL inhibited their expression in a dose-dependent manner. These findings suggest that WEL suppresses iNOS and COX-2 enzyme expression, which in turn reduces the production of NO and PGE2, two critical inflammatory mediators.

Effects of WEL on LPS-Mediated NF-κB Transcriptional Activity via Inhibition of IκB-α Degradation and Nuclear Translocation of p65 and p50 Subunits in RAW 264.7 Cells
NF-κB plays a central role in regulating the expression of iNOS, COX-2, and inflammatory cytokines like TNF-α [12]. The NF-κB complex, in its inactive form, is sequestered in the cytoplasm in association with the inhibitory protein IκB-α. Upon activation, NF-κB translocates to the nucleus, a process initiated by the phosphorylation, ubiquitination, and subsequent degradation of IκB-α. To explore the mechanism by which WEL inhibits iNOS and COX-2 expression in LPS-stimulated cells, we utilized a luciferase reporter assay to assess the impact of WEL on NF-κB-dependent reporter gene expression. RAW 264.7 cells were transiently cotransfected with a pNF-κB-luciferase reporter vector and stimulated with LPS (1 μg/ml) with or without WEL. The results demonstrated that WEL significantly reduced NF-κB luciferase activity induced by LPS in a dose-dependent manner .To further investigate WEL's influence on the NF-κB pathway, we assessed the cytoplasmic levels of IκB-α by western blotting after cells were pretreated with different concentrations of WEL for 12 hours and then stimulated with LPS (1 μg/ml) for 30 minutes. WEL treatment inhibited both the phosphorylation and degradation of IκB-α following LPS stimulation. Since p65 and p50 are key subunits of the NF-κB heterodimer, we examined their translocation from the cytoplasm to the nucleus after LPS treatment. As shown in Figures 5B and 5C, LPS treatment caused a decrease in the cytoplasmic levels of p65 and p50, while their levels increased in the nucleus. Pretreatment with WEL reversed this pattern in a dose-dependent manner. Taken together, these results indicate that WEL suppresses the expression of iNOS and COX-2, at least partially, through an NF-κB-dependent mechanism. [36]

Evaluation Tests for Wedelolactone-
Coumarins Test: The test was conducted following the method described by Thenmozhi et al. Four drops of the concentrated sample were placed near the edge of an aluminum oxide thin-layer chromatography (TLC) plate. The TLC plate was then placed in a jar containing a solvent system (Toluene:Acetone:Formic acid, 11:6:1), acting as the mobile phase. After the solvent had moved through the plate, the spot was examined under UV light. The presence of a purple to violet color indicated the presence of coumarins in the plant.
Flavones Test: The test was performed according to the method described by Jaffer et al. Ten grams of powdered explants were macerated in 95% ethanol and then filtered using Whatman No. 1 filter paper. A 10 ml aliquot of 50% ethanol was mixed with 10 ml of 50% aqueous KOH. The two solutions were combined, and the appearance of a yellow color indicated a positive result for flavones.
Phenol Compound Test: Test 1-The test was carried out following the method described by Thenmozhi

et al. Two milliliters of the filtered plant extract were mixed with 1% ferric chloride solution. The appearance of a dark blue color indicated the presence of phenolic compounds.
Zainab A. Shanshol, Mouruj A. Alaubydi, Ali Sadik, The Extraction and Partial Purification of Wedelolactone from Local Eclipta alba Plant, Iraqi Journal of Science, 2013, Vol 54, Supplement No.4, pp:1084-1089

Test 2- Total Phenol Estimation: To estimate total phenol content, 100 mg of the sample was dissolved in 1 ml of methanol. From this solution, 20 µl was mixed with 180 µl of water, followed by the addition of 0.5 ml Folin-Ciocalteu reagent, 0.5 ml water, and 1 ml of 7.5% sodium carbonate solution. The mixture was incubated for 2 hours, after which the absorbance was measured at 726 nm using a spectrophotometer. Gallic acid was used as the standard, and the results were expressed as Gallic acid equivalents.[5]

Conclusion

Natural fresh plant sources rich in phytochemicals and antioxidants hold great potential as valuable supplements in integrative medicine. This review provides a detailed overview of recent scientific studies documenting the therapeutic properties of Wedelolactone (WDL). It covers WDL's antibacterial, analgesic, antioxidant, cytotoxic, antidiabetic, anti-inflammatory, neuroprotective, cardioprotective, and hepatoprotective activities, along with the mechanisms underlying these effects.

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