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ANTI-INFLAMMATORY AND WOUND HEALING ACTIVITIES OF WEDELOLACTONE IN COMBINATION WITH BERBERINE IN ALBINO WISTAR RATS

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ABSTRACT

In present research investigation, research project was started with the extraction, isolation and purification of berberine from *Berberis aristata* using phytochemical investigations and concentration of berberine (BBN) was also found to be 0.2% (HPLC, RT- 5.04 min). Further, wedelolactone (WLN) from *Eclipta alba* Linn was extracted, isolated, (IPTLC method) purified, and characterized and its concentration was found to be 2.2% (HPLC). Both WLN and BBN were found to possess strong antioxidant properties (free radicals scavenger). In pharmacological investigations, rats given WLN had better edoema inhibition at the 5-hour mark after receiving the usual medication. High dose concentration of BBN demonstrated good anti-inflammatory effects. The combined effect of WLN and BBN was more potent in anti-inflammatory assessment (edema-inhibition). Wound healing investigations of WLN and BBN using the excision wound procedure showed significant wound contraction occurred on days 15 and 21 as a result of the combination of WLN and BBN which encouraged efficient epithelialization. Better wound contraction / wound-healing (restoration of normal architecture) was induced by the combination of WLN and BBN. The histology of the animals treated with WLN and BBN showed fibrosis with inflammatory cells, mainly lymphocytes and fibroblasts, a high rate of wound contraction, a marked increase in regenerated tissue, and rapid epithelialization (a reduction in the time required for

epithelialization).

KEYWORDS: Berberine, anti-inflammatory, edema, epithelialization, phyto-pharmaceuticals, secondary plant metabolites, wedelolactone, wound healing.

1. INTRODUCTION

1.1. Inflammation and Inflammatory Process

Shih *et al*., 2007, the term "inflamers" / "inflammatio" is sequence of events that occur in response to noxious stimuli, infection or trauma. Inflammation is pervasive term, which is elicited by human body in response to obnoxious stimuli (nonspecific defensive / immune system's response to a stimulus / tissue damage) as a protective measure. Inflammation is caused by microbial, autoimmune, allergic, metabolic and physical insults which produce different types of inflammatory responses (Fig.1-Fig.2). In inflammation (body's severe reaction to any damage; ubiquitous process which induced homeostasis such as damage, exposure to contaminants / infection and also triggered by innate immune system receptors).

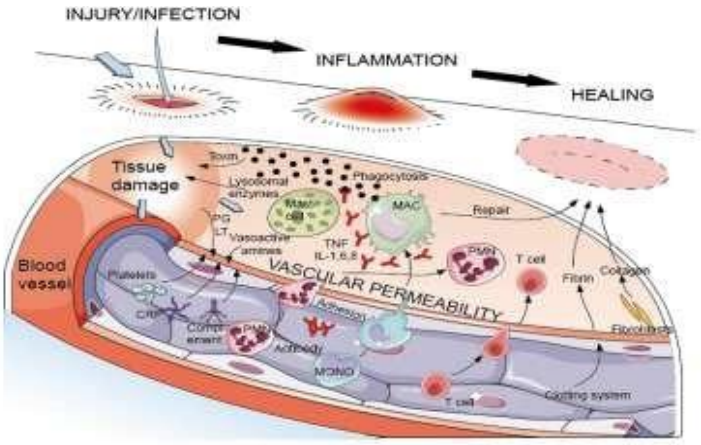

Fig. 1: Inflammation physiological process.

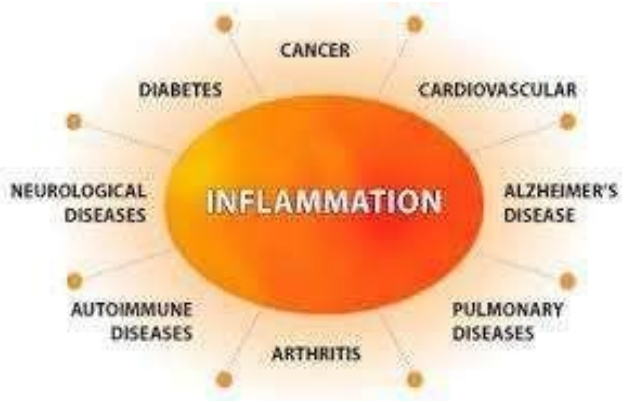

Fig. 2: Diseases associated with Inflammation.

Libby *et al.*, 2003, the prevalence of diseases with an inflammatory etiology or pathophysiology is rising. In addition to being expensive and infrequently available, synthetic medications used as anti-inflammatory treatments have harmful side effects. It has been helpful to research and produce anti-inflammatory medications. Effectiveness, affordability, accessibility, and minimal or nonexistent adverse effects are all factors that are showing promise. For appropriate prophylactic and therapeutic evaluation, the anti-inflammatory potential of these medicinal plants is being assessed both in vitro and in vivo, as well as their use in clinical and experimental settings. The use of medicinal plants to treat inflammatory illnesses has shown encouraging outcomes. To improve the assessment of active principles, new techniques for extracting and studying phytoconstituents from these therapeutic plants are being investigated.

1.2. Types of Inflammation

Roomme *et al.*, 2007, inflammation is either acute or chronic. Acute inflammation is an initial response of the body to harmful stimuli. In chronic inflammation, the response resulting in damage to the body (out of proportion; rheumatoid arthritis, asthma, colitis, allergies, hepatitis, metabolic syndrome, autoimmune diseases cancer, cardiovascular dysfunctions and neurodegenerative disorders**).** Inflammation is either acute or chronic type. Besides, on the basis of their cause inflammation may be microbial, autoimmune, allergic, metabolic, and physical inflammation. Acute inflammation increases vascular permeability, infiltration and emigration of leukocytes whereas chronic inflammation includes infiltration of mononuclear immune cells, macrophasaes, monocytes, neutrophils, fibroblast activation, proliferation and fibrosis (Fig.3).

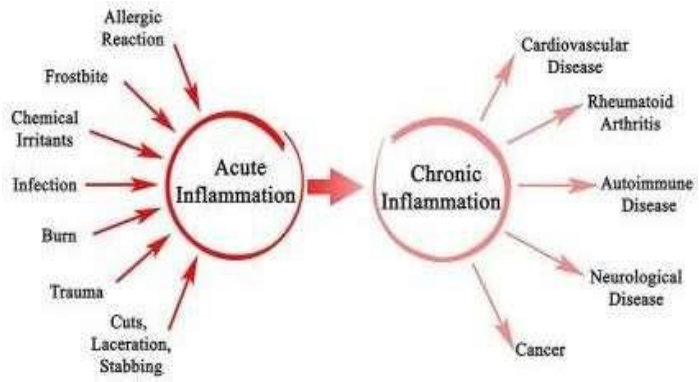

Fig. 3: Types of Inflammation.

1.3. Cause and Consequences of Inflammation

Inflammation disturb homeostasis and severe cause of morbidity (etiology or pathology).

Type of Agent	Example	Remarks
Biological	Virus, bacteria, fungal infections;	Acute inflammation (bacteria) Chronic inflammation (virus)
Chemical	Poisons and toxins	Inflammatory responses
Physical	Trauma (heat, burn, mechanical pressure etc.) causing tissue injury; Splinters (foreign body);	Acute to chronic inflammation
Immune reactions	Autoimmune diseases Immune responses to allergens	Chronic inflammation

1.3.1. Acute Inflammatory Response

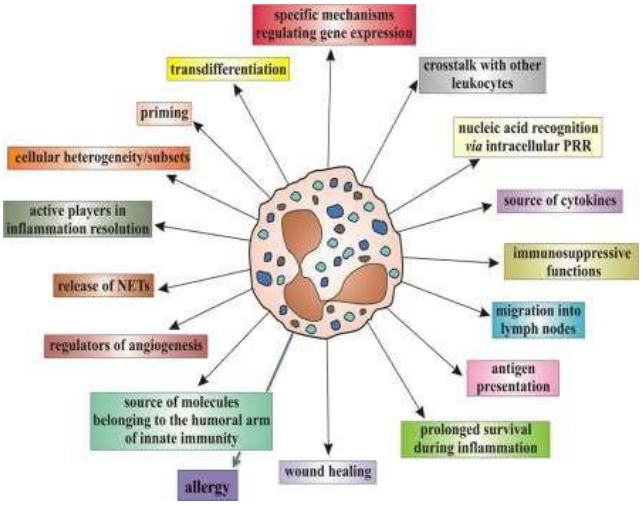

Fig. 4: Acute Inflammatory Response. (Marc *et al*., 2022)

1.4. Signs of Inflammation

Parhnam *et al*., 2008, inflammation is characterized by five classic inflammatory signs like redness, swelling, heat, pain and subsequent loss of organ function **(**signs of inflammation include redness (local), swelling, pain, heat and loss of function). Inflammatory responses are as follows:

i. Vasodilation (blood vessels increased permeability);

ii. Movement (Emigration) of phagocytes (blood to interstitial fluid);

iii. Tissue repair;

1.5. Pathophysiology of Inflammation

Dewanjee and colleagues (2013), cyclooxygenase (COX) is a key enzyme that contributes to the synthesis of prostacyclins, prostaglandins, and thromboxanes, which are implicated in pain, inflammation, and platelet aggregation **(Fig.5; Iwalewa *et al*., 2007)**.

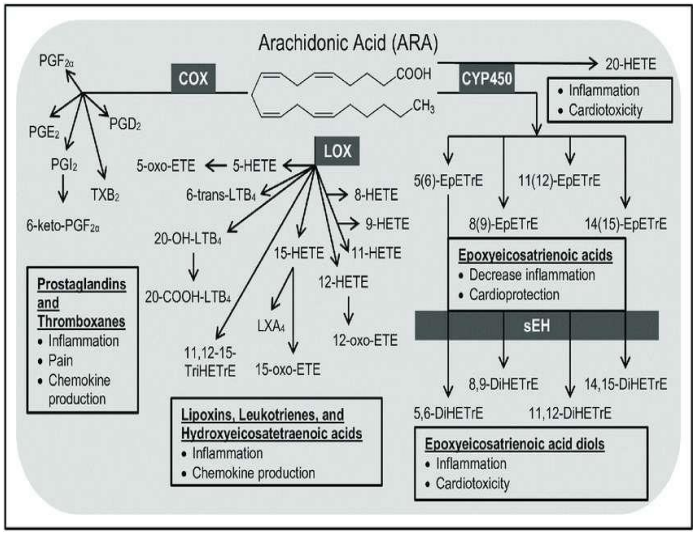

Fig. 5: Role of arachidonic acid (AA) pathway in inflammation.

1.6. *In-vivo* Screening of anti-inflammatory drugs (Fabian *et al.*, 2019)

Hunskaar *et al.*, 1986, evaluation by formalin test; **Koster *et al.*, 1959,** acetic acid induced vascular permeability tests**; Anuario *et al.*, 2018,** Oxazolone induced ear edema test**; Batista *et al.*, 2016,** carrageenan induced paw edema; **Da Silva *et al.*, 2018,** pleurisy test; **Calil *et al.*, 2014,** lipopolysaccharide induced edema test **(Moreno *et al.*, 1993; Al-Haboubi *et al.*, 1983; Coura *et al.*, 2015, Katz *et al.*, 1984; Table 1).**

Table 1: Assessment methods.

Test
➢ By formalin test
➢ Bradykinin induced paw edema
➢ Oxazolone induced ear edema
➢ Acetic acid induced vascular permeability
➢ Arachidonic acid induced ear edema
➢ Histamine induced paw edema
➢ Croton oil induced ear edema in mice
➢ Dextran induced edema
➢ Lipopolysaccharide induced paw edema
➢ Carrageenan induced paw edema
➢ Pleurisy tests

1.7 Treatment of Inflammation

1.7.1. Non-steroidal anti-inflammatory drugs (NSAIDs)

Pereira-Leite *et al.*, 2017, non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat inflammation (acute and chronic pain) by inhibition of COX-1 and COX-2 which stop accumulation of prostaglandins and thromboxanes. Disadvantage of NSAIDs is their toxicity

and reappearance of symptoms after discontinuation. Screening and development new anti-inflammatory drugs are the need of hour and efforts are made to find anti-inflammatory drugs from natural sources. **(Sostres and Lanas, 2016)**

1.7.2. Natural Products (NP) in the Treatment of Inflammation

Newman (2020), more than 80% of medicines have been developed from natural products (NP) obtained from natural source (NPs or pharmacophore from a NP). Biologically active compounds for many centuries and use extensively as crude material or as pure compounds for treating various disease conditions. India with its biggest repository of crude drugs or as bioactive compounds in the formulation of pharmaceuticals and cosmetics etc. **(Goel *et al.*, 2020)**.

1.8. Wound: Pathophysiology & Physiology of Wound Healing

Meenakshi *et al.*, 2006, an open wound is one in which the skin is torn, sliced, or punctured; a closed wound is one in which blunt force trauma results in a contusion. It is a reference to a sharp wound that harms the skin's dermis. The object that produces the wound can determine the classification of an open wound. Wounds types include a cut or incised wound brought on by a clean, sharpened tool, such a knife or razor. The wounds are irregular and resemble tears due to physical trauma. Grazes and abrasions are superficial wounds obtained by scraping off the epidermis, the topmost layer of skin. Puncture Wound: Resulting from an instrument, like a needle or nail, puncturing the skin. A penetration wound is when anything, like a knife, pierces or emerges from the skin. Categories of closed wounds include a blunt force trauma that destroys tissue beneath the surface is the cause of contusions, often known as bruises, damage to the blood vessel that results in the accumulation of blood beneath the skin is the cause of haematomas, also known as blood tumors and Extreme force applied over an extended period of time might result in crash injuries.

2.1. Wedelolactone

Neerja *et al.*, 2008, Wedelolactone (furanocoumarin; $C_{16}H_{10}O_7$; mol. wt. of 314.3; yellow-green solid) compound as a coumestan that occurs in *Eclipta alba* (false daisy) and in *Wedelia calendulaceae*. Wedelolactone (7-methoxy-5,11,12-trihydroxy-coumestan; Fig.6) possess hepatoprotective, sedative, muscle-relaxant, anxiolytic, anti-stress activities. **Thorat *et al.*, 2010,** it is present in *Eclipta alba* (Asteraceae; Fig.7), *Wedelia calendulacae* (Asteraceae), *Wedelia sinensis* (Asteraceae), *Eclipta prostata* (Asteraceae). The Wedelolactone can be found in the roots, leaves, stem and bark of the plants. **(Lal *et al.*,**

2010)

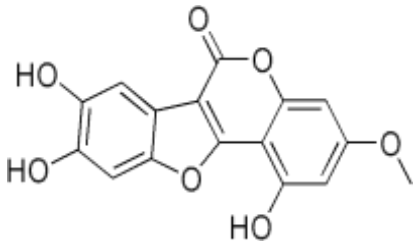

Fig. 6: Chemical Structure of Wedelolactone.

Fig. 7: *Eclipta alba* Linn Field Photograph.

2.2. Berberine

Xia *et al.*, 2010, berberine (Fig.8) is a bitter, yellow colored (shows a strong yellow fluorescence under UV light) quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids found in *Berberis Vulgaris* (Barberry), *Berberis aristata* DC (goldenseal; Fig.9), oregon grape, and goldthread. Berberine is used as dye, antibiotic against bacteria, viruses, fungi, protozoans, helminthes, and chlamydia, and in histology for staining heparin in mast cells.

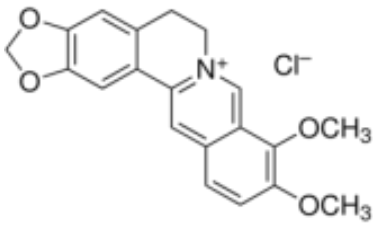

Fig. 8: Chemical Structure of Berberine.

Fig. 9: *Berberis aristata* DC.

3. AIM AND OBJECTIVES

Gessner *et al.*, 2017, the use of medicinal plants in inflammatory illnesses has produced encouraging outcomes. In this field, numerous patents have been obtained as a result. Prior to being used in clinical studies with cutting-edge technologies and study designs. The proposed study will be carried out with the following aims and objectives:

- To develop standardized protocols for extraction, separation and purification of the wedelolactone and berberine using modern scientific methods.
- To assess the dose dosing schedule and dose dependent studies of wedelolactone and berberine for anti-inflammatory and wound healing activities.

4. MATERIALS AND METHODS

4.1. Procurement and Authentication

Stem bark of *Berberis aristata* (tree turmeric) was procured from commercial source while and leaves of *Eclipta alba* (Bhringraj) was collected from plants grown in Herbal Garden situated in the campus of Pharmacy Department, IEC Group of Institution, Greater Noida Uttar Pradesh. Procured plant materials were assessed pharmacognostically for authentication to establish their identity. Herbarium specimens of *Berberis aristata* (IEC/ Pharm/Herb/ 2024/2401*)* and *Eclipta alba* (IEC/Pharm/Herb /2024/2402) were deposited.

4.2. Extraction and Isolation of Berberine

For eight hours, 1000 g of coarsely ground stem bark material was continuously heated and extracted using ethanol. Distillation was used to filter and concentrate the extract. To separate the resin (as an impurity), 82.4 g of residue was added to hot water and filtered while still hot. berberine was separated from the aqueous extract by treating it with an excess of

hydrochloric acid and letting it rest for 16 hours. 17.6 g of filtered berberine was refined by dissolving it in hot water and adding 10% alkali solution to make the solution alkaline. After adding acetone, the mixture was left to stand at 5°C for the entire night. Following separation and cold water washing, dried berberine was dissolved in 10:1 (alcohol and chloroform). For crystallization and recrystallization extract was boiled and then cooled to 5°C in petridishes.

4.2.1. Separation of Berberine by TLC

Step Method

(i) Preparation (Silica gel–G slurry) and activation of the TLC plates

(ii) Saturation of Mobile Phase Chromatographic Chamber

(iii) Application of the spots (with micro capillaries)

(iv) Development of chromatogram / Detection of spots

Dragendorff's spraying reagent (freshly prepared) was used and subsequently heated in an oven for few minutes. The R_f values of the visualized spots was calculated.

4.2.2. Quantitative estimation of Berberine by HPLC method

Technique	• Reverse phase HPLC
Eluting solvent	• Acetonitrile-methanol-0.05M tartaric acid (46:10:44)
Detector	• UV (260nm)
Flow Rate	• 1 ml/min.

4.3. Extraction and Isolation of Wedelolactone

In a Soxhlet apparatus, 1000 g of leaves were dried in the shade, roughly ground, and extracted using methanol. Evaporation is used to filter and concentrate the extract. Waxy substance was eliminated by suspending the concentrate in water and heating it on a water bath for 30 minutes below 80 degrees Celsius. Ethyl acetate is used to filter and partition the extract six times. Sodium sulfate is used to dry the ethyl acetate fraction.

6.8 g of light brown powder is produced after the solvent is evaporated. The extract undergoes silica-gel column chromatography. Fractions eluted using dichloromethane as the mobile phase: Crude is obtained from the collection of methanol: water: 14:9:4.

4.3.1. Isolation of Wedelolactone (WLN) from methanolic extract by IPTLC

Plate thickness	• 250 µm (wet) (Silica gel – G)
Solvent system	• Toluene: Acetone: Formic acid:: 11:6:1
Spraying Reagent	• Vanillin- Sulphuric acid spraying reagent

4.3.2. Preparation for Vanillin- Sulphuric acid: Spray reagent

1 gm of Vanillin + 90 ml methanol + 10 ml Sulphuric acid (Mixed very carefully)

Fluorescence	R_f Value
Green	0.64

4.3.3. Estimation of Wedelolactone by HPLC method

Technique	• Reverse phase HPLC
Eluting solvent	• Water: acetonitrile (65:35) with 0.1N Phosphoric acid
Detector	• UV (254 nm)
Flow Rate	• 1 ml/min.

4.4. *In-vitro* Antioxidant effects of Berberine and Wedelolactone

In this experiment, berberine and wedelolactone were analysed for their antioxidant properties by DPPH Analysis, NO Analysis, FRAP Assay, Reducing Power Assay **(Benzie and Strain, 1996; Jayaprakasha *et al*., 2001; Gutteridgde, 1995)**

4.5. *In-vivo* Anti-inflammatory Activity of Berberine and Wedelolactone

Anti-inflammatory studies (IAEC Form B approval : IEC/IAEC/2025/01 dated 17-05- 2025) of berberine and wedelolactone were carried out as *in-vivo* method reported by **Winter *et al.*, 1962**. The anti-inflammatory property was assessed by plethysmograph.

4.5.1. Animal Groups and Treatment Schedule

Table 2: Grouping of Animals for Anti-inflammatory Activity.

Group	Schedule	Animals
I	Normal (Water *ad libitum*)	6
II: Toxic	Subplantar injection of100 μL of 1% (1% w/v carrageenan)	6
III: Drug Treated	100 μL of 1% w/v carrageenan + WLN (50 mg/kg b.wt.)	6
IV: Drug Treated	100 μL of 1% w/v carrageenan+WLN (100 mg/kg b.wt)	6
V: Drug Treated	100 μL of 1% w/v carrageenan + BBN (50 mg/kg b.wt.)	6
VI: Drug Treated	100 μL of 1% w/v carrageenan +BBN (100 mg/kg b.wt.)	6
VII : Drug Treated	100 μL of 1% w/v carrageenan + combination of WLN (50 mg/kg b.wt.)) and BBN (50 mg/kg b.wt.))	6
VIII: Standard	100 μL of 1% w/v carrageenan + Diclofenac (Standard) (10 mg/kg b.wt.p.o)	6

For the investigation, 48 albino wistar rats in good health (weighing 200±20 g) were chosen. Following acclimation, the animals were split into 08 groups at random (Group I to VIII; 06 animals/group). Water was given freely to Group I (Normal), also known as the control group. Animals in Group II (Toxic Control) received intra-peritoneal injections of 1% carrageenan (freshly prepared solution of carrageenan in distilled water). Groups III and IV

(Wedelolactone; WLN; 50/100mg/kg) One percent carrageenan was injected to cause inflammation. Group V and VI received oral administration of Berberine (BBN; 50/100 mg/kg b.wt.). Group VII received oral administration of combination of Wedelolactone and Berberine (50 mg/kg) and BBN (50 mg/kg). Group VIII (Std.): 10 mg/kg/bw of Diclofenac.

4.6. Evaluation of Wound Healing Properties of Wedelolactone and Berberine

Wound healing studies (IAEC Form B approval: IEC/IAEC/2025/01 dated 17-05- 2025) of WLN and BBN by excision wound method were carried out as method reported by **Morton and Malone (1972)**.

4.6.1. Grouping of Animals (Six animals/group; Table 3)

Table 3: Grouping of Animals for wound healing activity.

Group	Dosage Treatment
I	Control
II	Toxic
III	WLN (100 mg/kg body weight)
IV	BBN (100 mg/kg body weight)
V	Combination of WLN (50 mg/kg body weight) and BBN (50 mg/kg body weight)
VI	Soframycin Ointment (Standard)

4.6.2. Procedure

(i)	Animals were anaesthetized with ketamine + Xylazine.
(ii)	An excision wound (5cm; ring shaped) was made on dorsal thoracic region (depilated ethanol-sterilized) of male rats.
(iii)	WLN, BBN and combination of WLN and BBN were given to different group of animals (group III-V).
(iv)	Subsequently, wound contraction was analysed by tracing the raw wound area on day 1, 4, 8, 12, 16, 18 and 21 using graph paper.
(v)	Assess wound healing, scar area, epithelialisation.
(vi)	Further, wound closure (%) and epithelialisation period was recorded.

RESULTS AND DISCUSSION

The acquisition of raw materials marked the beginning of the dissertation research project. *Berberis aristata* DC stem bark was purchased from a commercial source, and *Eclipta alba* leaves were gathered from plants cultivated in the Institute's medicinal plant garden. Using morphological, microscopical, and chemical pharmacognostical evaluation techniques, it was verified. Additionally, a phytochemical screening was conducted to find wedelolactone in the leaves of *Eclipta alba* Linn. and berberine in the stem bark of *Berberis aristata* DC. Herbarium specimens were placed in the Institute's herbarium bank. *Berberis aristata*

(IEC/Pharm/Herb/2024/2401) and *Eclipta alba* (IEC/Pharm/Herb/2024/2402).

Following that, phytochemical studies were conducted to extract, isolate, purify, and quantify berberine. Phytochemical experiments were conducted using standardized procedures. The crystals of BBN obtained were golden yellow with m.p. 145^{o}C. The compound BBN contains multiple polar groups and so found to be easily soluble in methanol, ethanol, and acetone while slightly soluble in hot water. Alkaloidal salt of BBN was soluble in water and relatively poorly soluble in organic solvents. Isolated berberine is an isoquinoline alkaloid and its crystals were yellow golden color which showed yellow fluorescence in ultraviolet light. The chromatographic analyses confirmed that the isolated and purified compound was berberine.

Fig. 10: TLC Chromatogram of Berberine.

Using Silica gel-G as the stationary phase and n-Butanol-acetic acid-water (7:1:2) as the mobile phase, berberine was analyzed by TLC. Eluted TLC plates were created by spraying them with freshly prepared Dragendorff's spraying reagent, and the Rf values of the brown spots that were visible were 0.590 (Fig.10).

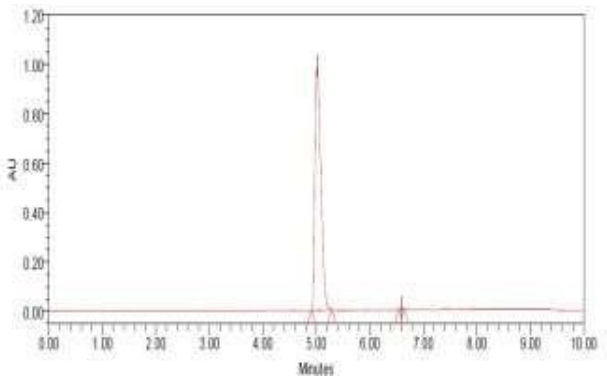

Fig. 11: HPLC Chromatogram of isolated and purified Berberine.

The isoquinoline alkaloid berberine was isolated, and its crystals were yellow-gold in color (crystallized and re-crystallized), with a melting point of 145^{0} C and yellow fluorescence when exposed to UV light. Berberine concentration was determined to be 0.2% in HPLC, and separation was accomplished in 5.04 min (Fig.11).

The next stage of the research project involved conducting phytochemical research to extract, isolate, purify, and characterize wedelolactone from *Eclipta alba* Linn. The resulting wedelolactone crystals were brown-beige in hue and readily soluble in hot water, methanol, and DMSO. Wedelolactone was isolated using the IPTLC method. Wedelolactone was identified as the ingredient by the isolated compound's Rf value. WLN concentration was determined to be 2.2% in HPLC, and separation was accomplished in 4.85 min (Fig.12).

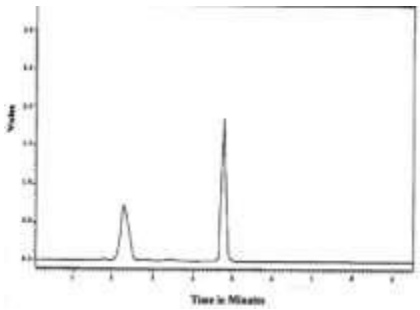

Fig. 12: HPLC chromatogram of purified wedelolactone.

Ascorbic acid, wedelolactone, and berberine were discovered to have very strong antioxidant properties. Wedelolactone and berberine have demonstrated the ability to scavenge free radicals. The amount of TPCs (polyphenols) in wedelolactone and berberine was directly correlated with their capacity to reduce ferric ions. According to the FRAP assay, berberine and wedelolactone have highly substantial antioxidant activity.

Table 4: DPPH regression curve.

Conc.(µg/mL)	The cleared ratio (%)		
	WLN	BBN	Ascorbic Acid
0	0	0	0
10	12.4	14.4	14.4
20	23.2	24.6	25.2
40	33.8	35.2	35.8
60	42.6	45.4	46.4
80	52.8	55.4	57.4
100	62.4	65.8	68.2

Anti-oxidant effects of the BBN, WLN and ascorbic acid were found to very high. BBN and WLN have shown free radical scavenging activity (Table 4; Fig.13). Antioxidant activity of BBN and WLN by FRAP assay was found to be very significant.

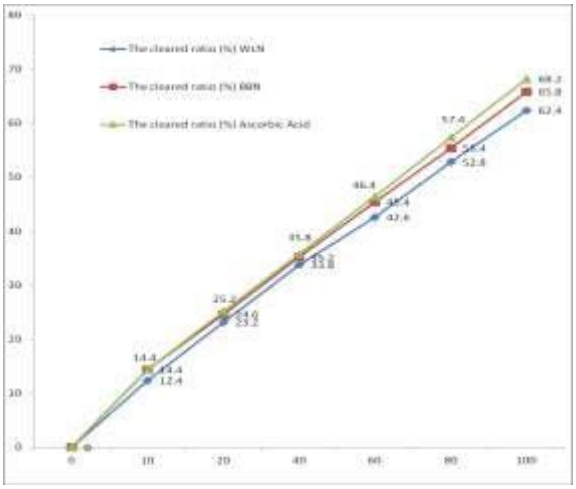

Fig. 13: The regression curve of DPPH.

In-vivo anti-inflammatory investigations of BBN and WLN were conducted using the IAEC Form B approval: IEC/IAEC/2025/01 dated 17-05-2025. Group I (Normal), usually referred to as the control group, received free water. 1% carrageenan was injected intraperitoneally into the animals in Group II (Toxic Control). Groups III and IV (WLN: 50/100 mg/kg b.wt.) and to induce inflammation, 1% carrageenan was administered. Berberine was administered orally to Groups V and VI at a dose of 50/100 mg/kg b.w. Wedelolactone (50 mg/kg b.wt.;) and berberine (50 mg/kg b.wt.;) were administered orally to Group VII. Group VIII (Std.): Diclofenac 10 mg/kg/bw.

Table 5: Effect of WLN and BBN in rat edema.

Group	edema / Time (Hr)				
	1	2	3	4	5
I-Normal	--	--	--	--	--
II-Toxic	1.64±0.14	1.72±0.12	1.86±0.18	1.92±0.28	1.98±0.18
III : WLN	1.64±0.24	1.56*±0.16	1.52*±0.12	1.48*±0.16	1.42*±0.12
IV : WLN	1.66±0.22	1.54*±0.14	1.50*±0.14	1.46*±0.14	1.38*±0.16
V : BBN	1.64±0.26	1.48*±0.18	1.44*±0.16	1.42*±0.12	1.38*±0.12
VI :BBN	1.60*±0.12	1.44*±0.12	1.42*±0.10	1.38*±0.14	1.34*±0.14
VII: WLN + BBN	1.52*±0.14	1.42*±0.16	1.40*±0.12	1.34±0.12	1.30*±0.16
VIII: Standard (Diclofenac;10mg/kg)	1.50*±0.16	1.40*±0.12	1.36*±0.16	1.32*±0.20	1.28*±0.18

Note: *significant (P<0.05)

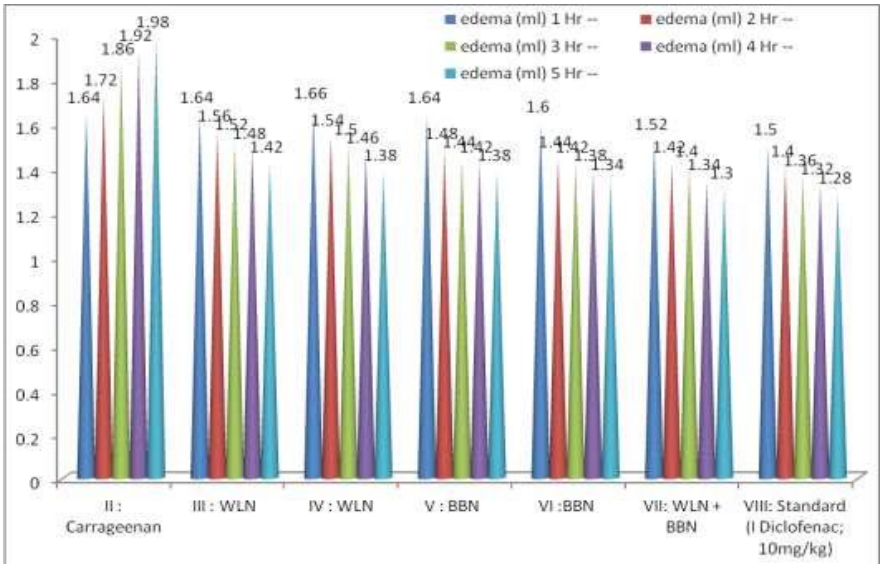

Fig. 14: Effect of WLN and BBN on paw edema.

Table 6: Wedelolactone, Berberine and standard induced inhibition of edema.

Group	edema inhibition (%)				
	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr
III : WLN (50 mg/kg)	5.72	16.52	18.96	22.56	24.86
IV : WLN (100 mg/kg)	10.38	14.92	19.92	24.26	30.58
V : BBN (50 mg/kg)	5.86	16.58	20.28	23.34	26.98
VI : BBN (100 mg/kg)	11.48	18.46	21.84	26.68	33.62
VII : WLN (50 mg/kg) + BBN (50 mg/kg)	12.84	19.16	22.14	27.04	36.14
VIII : Diclofenac (10mg/kg)	13.24	19.30	22.22	27.14	36.44

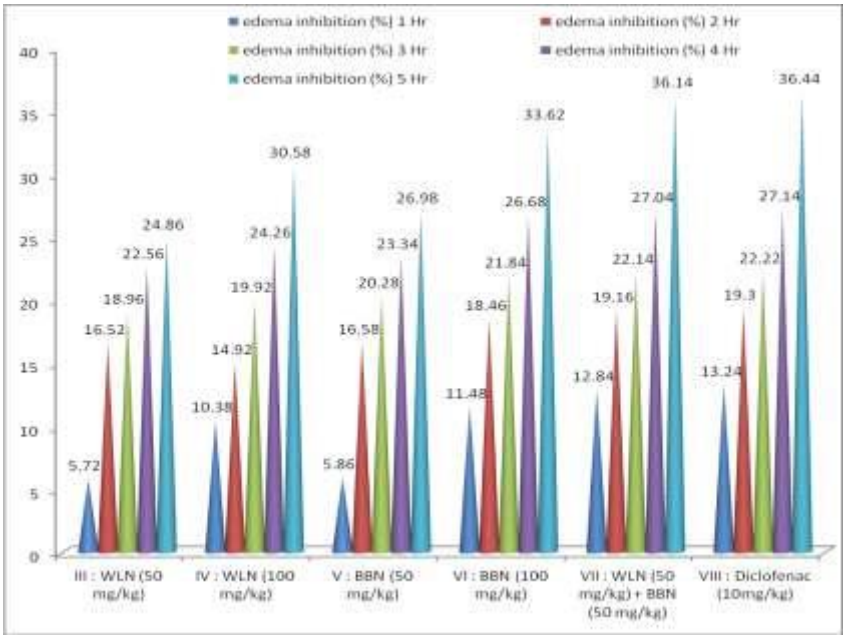

Fig. 15: Effect of WLN, BBN, standard drug on edema inhibition.

Following treatment with the standard medicine, rats treated with wedelolactone (WLN) showed improved edoema inhibition at the 5-hour observation. At high dose, berberine (BBN) produced anti-inflammatory effects. Combination of wedelolactone and berberine produced significant anti-inflammatory effects (edema inhibition). Besides, anti-inflammatory effects were less significant than standard reference drug i.e. diclofenac. Rats given WLN had better edoema inhibition at the 5-hour mark after receiving the usual medication (Table 5; Fig.14). BBN had anti-inflammatory properties at high doses. WLN and BBN together had strong anti-inflammatory (edema-inhibition) effects. Additionally, the anti-inflammatory effects were not as strong as those of the common reference medication, diclofenac ((Table 6; Fig.15).

As stated, wound healing tests of WLN and BBN using the excision wound procedure (IAEC Form B approval: IEC/IAEC/2025/01 dated 17-05-2025) were conducted. Significant wound contraction occurred on days 15 and 21 as a result of the combination of WLN and BBN which promoted effective epithelialization. In addition, group I control rats produced fibroblasts, neutrophils, lymphocytes, and necrotic debris. Combination of WLN and BBN produced good epithelialization which lead to significant wound contraction on day 15 and day 21. Besides, control rats (group I) induced necrotic debris, lymphocytes, neutrophils and fibroblasts. Soframycin showed good wound contraction ($p>0.05$; 33 % (4)→49.30% (8)→ 72.56% (12) and finally 95.62% (15). Combination of WLN (50 mg/kg body weight) and BBN (50 mg/kg body weight) showed good wound contraction ($p>0.05$; 30.62% (4^{th} day) → 44.24% (8^{th} day)→67.6% (12^{th} day) and finally 87.6 % (15^{th} day). The ability of combination of WLN and BBN to cure wounds was similar group V (restored normal architecture). In the case of the animals treated with WLN and BBN, the histology revealed rapid epithelialization (a reduction in the time required for epithelialization), a high rate of wound contraction, a marked increase in regenerated tissue, fibrosis containing inflammatory cells primarily lymphocytes and fibroblasts. WLN and BBN together had comparable wound-healing effects to group V (restored normal architecture). The histology of the animals treated with WLN and BBN showed fibrosis with inflammatory cells, mainly lymphocytes and fibroblasts, a high rate of wound contraction, a marked increase in regenerated tissue, and rapid epithelialization (a reduction in the time required for epithelialization).

CONCLUSIONS

In present research investigation, an attempt to conduct wound healing activity and anti-

inflammatory investigation of wedelolactone and berberine (either alone or in combination). Both WLN, and BBN were found to possess strong antioxidant properties (ability to scavenge free radicals). According to the FRAP assay, BBN and WLN have highly substantial antioxidant activity. In pharmacological investigations, rats given WLN had better edoema inhibition at the 5-hour mark after receiving the usual medication. The combined effects of WLN and BBN were potent anti-inflammatory (edema-inhibition). Wound healing investigations of WLN and BBN using the excision wound procedure showed significant wound contraction occurred on days 15 and 21 as a result of the combination of WLN and BBN which encouraged efficient epithelialization.

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