Pharmacological Assessment of *Cassia Occidentalis* Leaves for Anti-Inflammatory and Wound Healing Properties

[1]Ritesh Rai, [2]Sonia Bhatt, [3]Amrita Singh, [4]Vikash Kumar, [5]Bhanu P. S. Sagar

IEC Department of Pharmacy
IEC College of Engineering & Technology
IEC Group of Institutions, Greater Noida
Gautam Budha Nagar, Uttar Pradesh, India.

Abstract- **In present research investigation, an attempt to conduct wound healing activity and anti-inflammatory investigation of *Cassia occidentalis* L. *Cassia occidentalis* L. plant was scientifically authenticated. PPMs and SPMs like anthraquinones, phenolics, flavanoids, terpenoids and saponins were present. Percolation method yielded MECOL extract (3.8% yield) and EECOL extract (4.32% yield). Further, chrysophanol was purified by IPTLC method (10.6% in MECOL extract). In toxicity assessment, no abnormal behaviour; no significant alterations in hematological parameters, modification in renal function and LFTs even at higher dose. No mortality was found and EECOL, MECOL and chrysophanol were found be non-toxic constituent even at 2000 mg/kg/b.wt. (possess wide safety margin). LD50 was found to be 1000 mg/kg b.w. (chrysophanol) and 1600 mg/kg b.w. (MECOL / EECOL). Chrysophanol MECOL, and EECOL Extract produced significant anti- inflammatory effects and edema inhibition was maximum with standard drug (35.97%) followed by Chrysophanol (32.4%), MECOL-B (31.78%) and EECOL-B(31.22%). Treatment with MECOL-B, EECOL-B extract and chrysophanol produced significant wound healing comparable to soframycin ($p<0.01$). Histology of MECOL, EECOL and Chrysophanol treated animals showed high rate of wound contraction, increased epithelialisation and regenerated tissue, and new blood vessel formation. Finally, much more elaborative phytochemical and pharmacological research work is proposed to endorse the findings of the present research work.**

Keywords: **Anti-inflammatory, Chrysophanol, *Cassia occidentalis,* Kassaumdhi, percolation, Indomethacin, Carrageenan, physico-chemical analysis.**

Inflammatory Diseases

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 different types of inflammatory responses.

Causes of Inflammation

Table 1 : Various causes of Inflammation.

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

Inflammatory responses are as follows :

i. Vasodilation (blood vessels increased permeability);
ii. Movement (Emigration) of phagocytes (blood to interstitial fluid);
iii. Tissue repair;

Sign of Inflammation

Inflammation is characterized by different signs as follow:

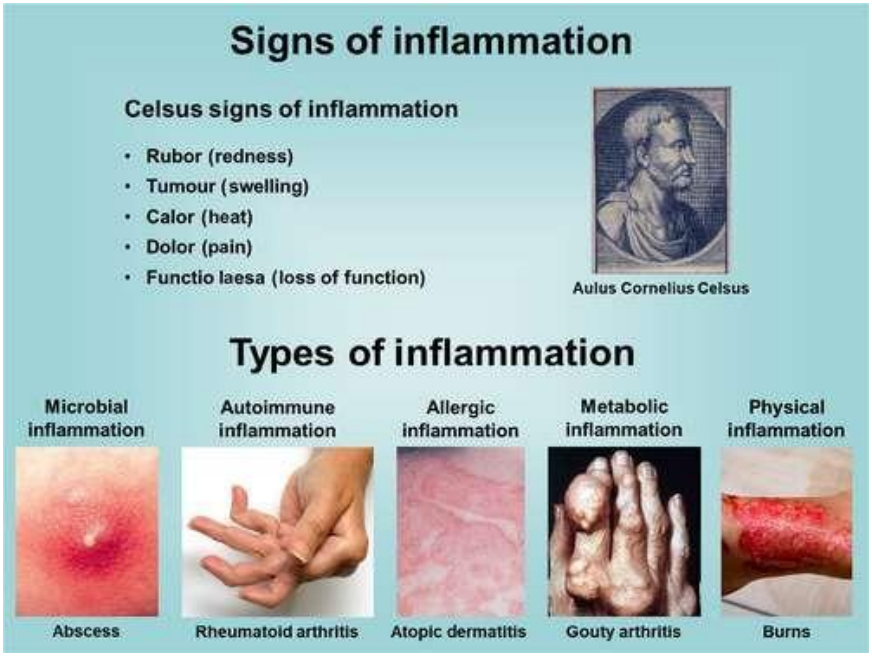

Figure 1 : Sign and Types of Inflammation

Types of Inflammation

Inflammation is either acute or chronic type. Besides, on the basis of their cause inflammation may be microbial inflammation (abscess), autoimmune inflammation (rheumatoid arthritis), allergic inflammation (atopic dermatitis), metabolic inflammation, (gouty arthritis) and physical inflammation (burns).

Acute Inflammation: Increased vascular permeability, infiltration and emigration ofleukocytes (Kumar, 2013).

Chronic inflammation: Infiltration of mononuclear immune cells, macrophasaes, monocytes, neutrophils, fibroblast activation, proliferation and fibrosis (Kumar, 2013);

Wounds : Pathophysiology and Healing

Wound is a breakage in the body tissues (physical injury) produced by physical (pressure ulcers), thermal (burns), mechanical (cut, abrasion, lacerations), chemical or immunological damage to the tissue.

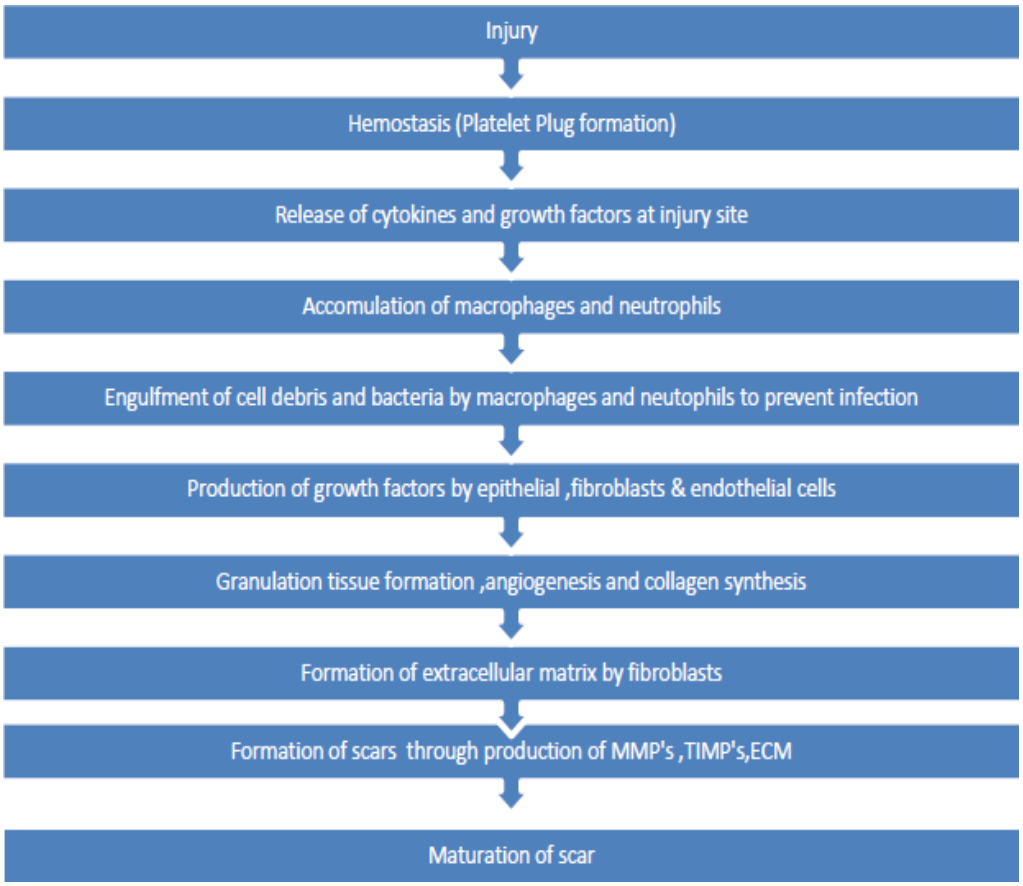

Figure 2: Physiology of Wound Healing.

***Cassia occidentalis* Linn.** (Ajagbonna *et al*., 2001)
Common Name: Kasmard, Kasondi, Coffee Senna, *Fetid cassia*, Negro Coffee

Manikandaselvi *et al., 2016, Cassia occidentalis* Linn. belongs to genus Cassia and is a annual or perennial weed plant (family : Fabaceae / Caesalpinoideae) found in South India, North, North-west India and across the world (Yadav *et al*., 2010; Isaac *et al*., 2016; Figure 3-5).

Pharmacognosy of *Cassia occidentalis* Linn. (Manikandaselv *et al*., 2016)

Figure 3: Field Photograph of *Cassia occidentalis* Linn.

Figure 4 : Photograph of flowers of *Cassia occidentalis* Linn.

Figure 5 : Photograph of leaves of *Cassia occidentalis* Linn.

Chemistry of *Cassia occidentalis* Linn.

Jawahar *et al*., 1974, phytochemical screening showed the presence of carbohydrates, saponins, sterols, flavonoids, resins, alkaloids, terpenes, anthraquinones, glycoside and balsam.(Kudav & Kulkarni, 1974; Tsutomu *et al*., 1999).

Pharmacological uses of *Cassia occidentalis* Linn.

Table 2: Pharmacological uses of *Cassia occidentalis* Linn.

PharmacologicalActivity	Reference
Immunosuppression	Bilal *et al*., 2001;
Antimicrobial	Vedpriya *et al*., 2010;
Antioxidant / hepatoprotective	Bhattacharyya *et al*., 2003; Jafri *et al*., 1999; Sadique *et al*., 1987;
Larvicidal	Abirami *et al*., 2011; Lienard *et al*., 1993;
Anti-malarial	Tona *et al*. 2001; Tona *et al*., 1999; Tona *et al*., 2004
Antidiabetic	Laxmi *et al*., 2010;

The scientific research on *C. occidentalis* suggested a huge biological potential of this plant. Chrysophanol was isolated from *Cassia occidentalis* for the study of wound healing and anti-inflammatory activities.

Materials and Methods

Procurement and Authentication of *Cassia occidentalis* Linn.

Fresh leaves of Kasondi (*Cassia occidentalis* Linn.; family : Fabaceae / Caesalpinoideae) were harvested locally from the college of the Institute during September – October 2022. Harvested Kassaumdhi leaves were checked morphologically, anatomically and chemical analysis to establish its identity with quality and purity. Herbarium specimenIEC/Pharm/Herb/2022/2210 was deposited for official records.

Qualitative analysis of Kassaumdhi Leaves.

The petroleum ether, chloroform, methanol and aqueous (polar and non- polar solvent) extracts of kasondi leaves (dried under shade, coarsely pulverized separately) of *Cassia occidentalis* L. (Kasondi) were subjected to preliminary phytochemical screening for PPMs and SPMs (chemical groups) using standard procedures described by Harborne (1973); Trease and Evans (1985); Sofowora (1993); Khandelwal (2008); Kokate (2005). Qualitative analysis of Kassaumdhi confirmed chemical groups (PPMS and SPMs) like phenolics, steroids, alkaloids, anthocyanosides, saponins, resins, tannins, proteins, cardiac glycosides, anthroquinone, terpenes, phlobatannins, balsams, amino acids, flavonoids, carbohydrates, and reducingsugars.

Physico-chemical Analysis

Freshly procured, washed, air-dried and pulverised / powdered leaves of kasondi was subjected to physico-chemical analysis forparameter like extractive values, ash values, fluorescence analysis in various solvents (visible light / long and short wavelength) etc. (Table 3-4)

Table 3 : Physicochemical analysis of leaves of *Cassia occidentalis* L.

Quantitative parameter	Values (%) w/w)
Alcohol Soluble Extractive Value (ASEV; mg/g)	180.8±20
Water Soluble Extractive Value (WSEV; mg/g)	300.8±9.6
Total Ash Value (TAV; %w/w)	15.42
Water Soluble Ash Value (WSAV; %w/w)	5.29
Acid Soluble Ash Value (ASAV; %w/w)	5.59
Pet-ether soluble Extractive Value (PSEV; mg/g)	76.0±0.8
Moisture content (%w/w)	69.81±0.31

Table 4: Fluorescence of Kasondi leaves.

Solvent	Observation		
	Visible Light	Long Wavelength	Short Wavelength
Benzene	Yellowish	Green	Red Dark
Pet-ether	Yellowish	Green	Red Dark
Acetone	Dark Green	Purplish	Dark Blue
Ethanol	Black	Red	Black
Water (Distilled)	Yellow	Light green	Yellow

Preparation of EECOL and MECOL Extract of Kasondi Leaves

Freshly harvested kasondi leaves shade dried, coarsely powdered. 1000 g leaves (pulverised) extracted separately with methanol / ethanol (w/v 1:3) by percolation for 01 week (repeated 03 times). Methanol crude extract fractions pooled and concentrated (47.5 g). Ethanol crude extract fractions combined and concentrated (53.8 g). Partitioning (solvent - solvent extraction:: nonpolar-polar solvent). Methanol extract : Chloroform fraction (9.5 g). methanol soluble fractions (38 g; 3.8%);Ethanol extract: chloroform fraction (10.6 g); ethanol soluble fraction (43.2 g; 4.32%). Practical yield EECOL and MECOL extracts were 4.32% and 3.8% respectively. Different concentrations of EECOL and MECOL extracts (200 / 400mg in 1 % carboxymethylcellulose) were used in pharmacological studies.

Isolation of Chrysophanol from EECOL and MECOL extracts

EECOL and MECOL extracts were subjected to instant preparative thin layer chromatography (IPTLC). Stationary phase: Silica gel-G. Mobile phase solvent system : chloroform: methanol (9:1). Spraying Reagent : 5% alcoholic NaOH / exposure to ammonia vapor. Chrysaphanol (anthraquinone) spots: Pink colour spots (R_f value: 0.88). Isolated chrysophanol was found to be an anthracene derivative, hydrophobic compounds, golden yellow crystalline powder; mol. formula $C_{15}H_{10}O_4$; mol. wt. 254.2; m.p.196^{0}C; UV λmax 277; soluble in methanol; poorly soluble in water; practical yield : 10.6%.

Safety and Toxicity Evaluation

Toxicity studies of EECOL, MECOL and Chrysophanol were carried out as per OECD by guidelines and IAEC Form B approval and animals were procured from CAHF, JNU, Delhi.

Acute and Sub-acute Toxicity Study

Physiological and behavioural changes were observed then RBC count, MCV, MCH, Hb content, hematocrit (Ht), WBC were analysed and diagnostic kits were also used for biochemical analysis (Table 5-7; Figure 6-7). EECOL, MECOL and Chrysophanol were administered physical and biochemical changes were observed. Group I animals served as normal control while Group–II, III and IV received different doses of EECOL, MECOL and Chrysophanol respectively and physiological and behavioural changes observed over a period of 72 hours.

Table 5: Toxicity (mortality) of EECOL, MECOL and Chrysophanol.

S. No.	EECOL (mg/kg.b.wt.)	MECOL (mg/kg.b.wt.)	Chrysophanol (mg/kg.b.wt.)	Percent mortality (%)
1.	50	50	50	No Mortality / Zero Mortality
2.	100	100	100	
3.	250	250	250	
4.	500	500	500	
5.	750	750	750	
6.	1000	1000	1000	
7.	1250	1250	1250	
8.	1500	1500	1500	
9.	1750	1750	1750	
10.	2000	2000	2000	

Table 6: EECOL, MECOL and Chrysophanol effects on blood parameters.

Parameter	Control Group	Groups		
		EECOL	MECOL	Chrysophanol
RBC Count	8.16 ± 0.35	8.82 ± 0.87	8.92 ± 0.68	9.17 ± 1.14
Hemoglobin (Hb)	14.24 ± 092	14.72 ± 0.86	14.86 ± 0.32	14.81 ± 0.98
Hematocrit (Ht)	49.31 ± 2.45	51.63 ± 3.34	52.62 ± 3.14	51.87 ± 3.62
MCV	51.11 ± 5.04	51.04 ± 4.32	51.84 ± 4.62	52.14 ± 5.24
MCH	17.86 ± 1.22	18.12 ± 2.21	18.54 ± 2.26	18.72 ± 1.62
Platelet Counts	805.2 ± 68.72	814.4 ± 58.74	818.6 ± 62.82	828.44 ± 66.32
WBC Count	7.18 ± 1.32	7.34 ± 1.38	7.42 ± 1.28	7.56 ± 1.46
Neutrophils Count	23.52 ± 2.13	23.64 ± 3.74	23.82 ± 3.24	23.96 ± 3.46
Eosinophils Count	1.36 ± 0.54	1.42 ± 0.62	1.48 ± 0.74	1.54 ± 0.62
Basophils Count	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocyte Count	69.72 ± 6.53	67.86 ± 6.54	68.26 ± 6.78	69.24 ± 5.84

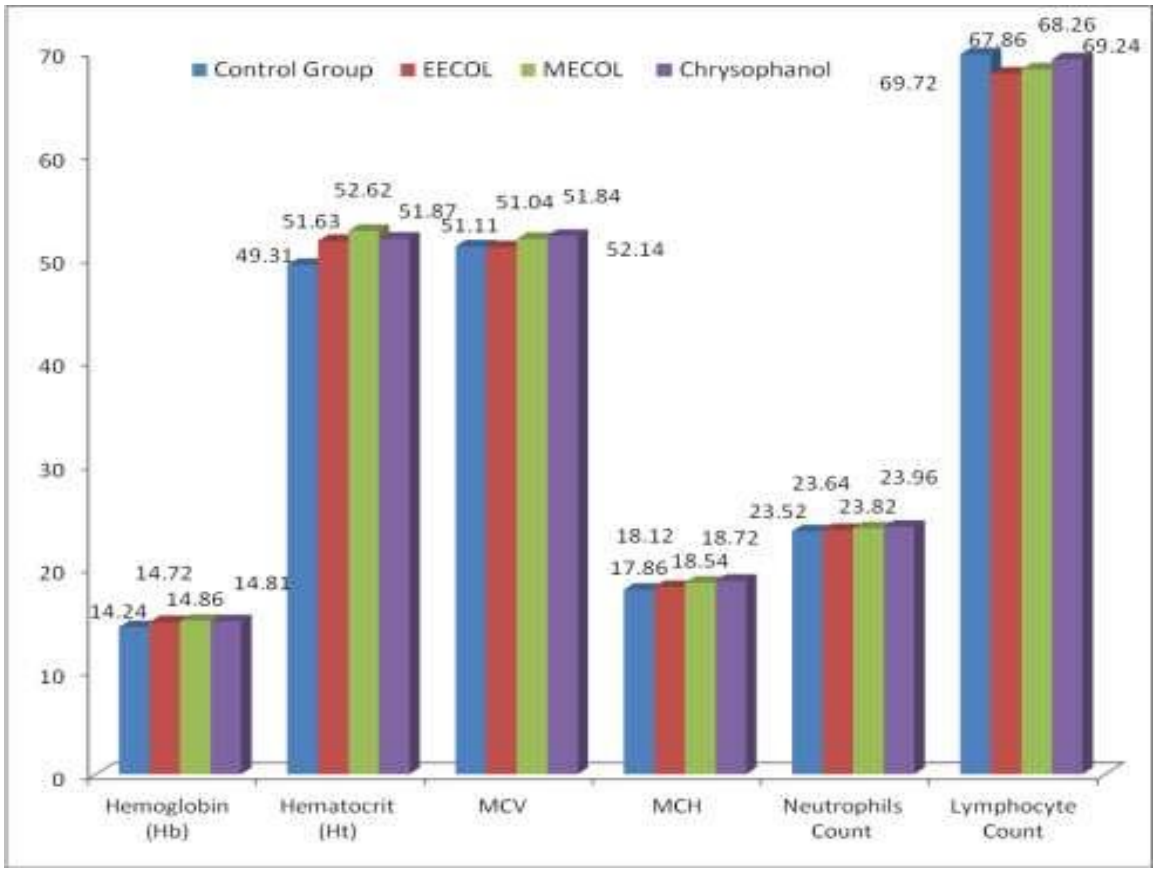

Figure 6: Effects of EECOL, MECOL and Chrysophanol on blood parameters.

Table 7: Effects of EECOL, MECOL and Chrysophanol on blood & LFTs.

Parameter	Control Group	Groups		
		EECOL	MECOL	Chrysophanol
Glucose	71.72 ± 6.24	63.68 ± 7.56	66.42 ± 8.44	68.28 ± 7.86
Creatinine	0.84 ± 0.12	0.86 ± 0.14	0.90 ± 0.08	0.94 ± 0.08
BUN	19.18 ± 1.62	18.86 ± 1.32	18.56 ± 1.24	18.46 ± 1.28
Tbil	0.34 ± 0.04	0.36 ± 0.06	0.38 ± 0..06	0.40 ± 0.08
SGPT	34.62 ± 4.28	34.62 ± 4.42	36.26 ± 4.18	40.26 ± 4.14
SGOT	48.6 ± 3.54	48.68 ± 2.64	50.42 ± 2.12	51.12 ± 2.52
AKLP	17.14 ± 4.42	17.34 ± 4.12	17.54 ± 4.36	18.24 ± 4.44
TC	54.86 ± 5.72	53.62 ± 5.36	55.14 ± 4.64	56.24 ± 4.76
T-Prot.	4.62 ± 0.26	4.60 ± 0.22	4.64 ± 0.48	4.68 ± 0.34
ALB	3.14 ± 0.08	3.16 ± 0.04	3.18 ± 0.04	3.20 ± 0.06

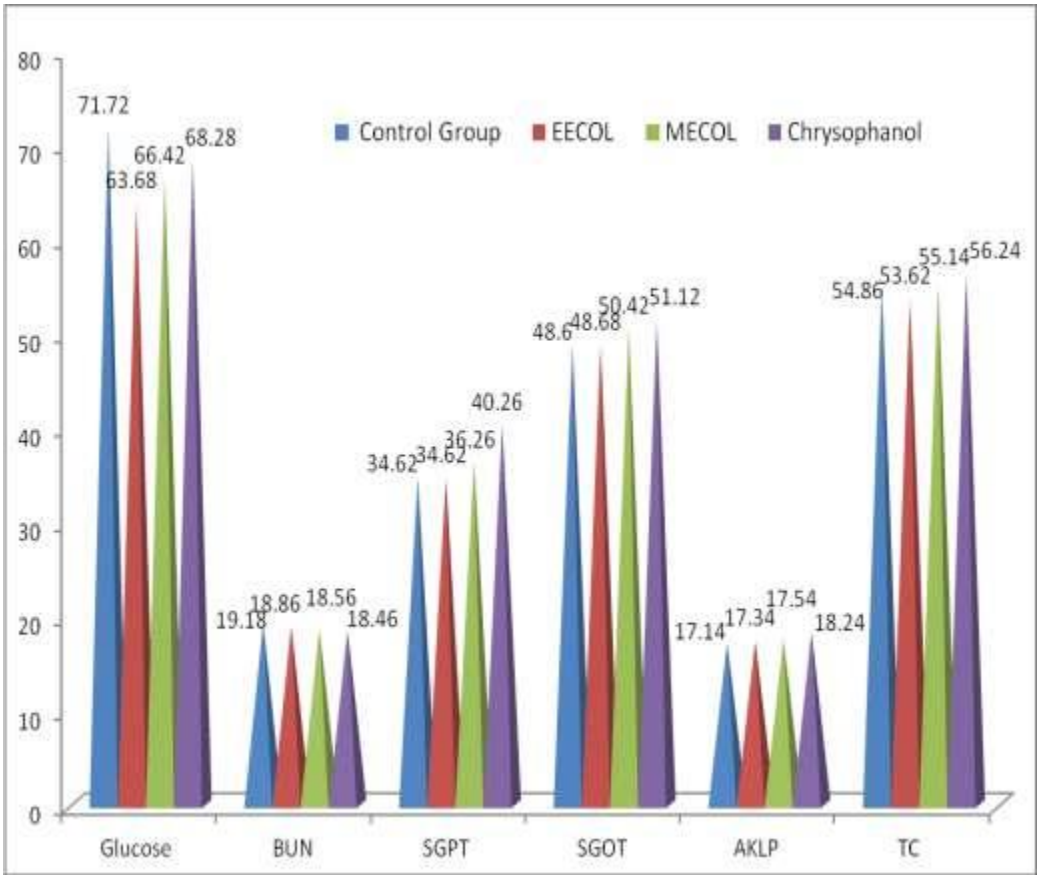

Figure 7: Effects of EECOL, MECOL and Chrysophanol on LFTs.

***In-vivo* Anti-inflammatory Activity**

Anti-inflammatory studies (IAEC Form B approval : IEC/IAEC/2023/04; 17-02-2023) of EECOL and MECOL extracts of Kasondi were carried out as *in-vivo* method (06 animal / group; Table 8-9; Figure 8-9).

Table 8: Effect of EECOL, MECOL and Chrysophanol on edema (1-5 hrs)

Group	Paw edema volume (ml)				
	1	2	3	4	5
I : Normal	--	--	--	--	--
II : (Toxic / Carrageenan)	1.78±0.018	1.80±0.024	1.86±0.062	1.90±0.038	1.88±0.024
III : EECOL-A (200 mg/kg)	1.68±0.05	1.46*±0.06	1.52*±0.06	1.44*±0.18	1.40*±0.02
IV : EECOL-B (400 mg/kg)	1.54*±0.06	1.40*±0.05	1.52*±0.07	1.44*±0.07	1.30*±0.10
V : MECOL-A (200 mg/kg)	1.62±0.05	1.42*±0.06	1.48*±0.06	1.40*±0.18	1.36*±0.02
VI : MECOL-B (400 mg/kg)	1.52*±0.06	1.38*±0.05	1.46*±0.07	1.38±0.07	1.34*±0.10
VII : 50 mg Chrysophanol	1.54*±0.09	1.44*±0.04	1.48*±0.066	1.36*±0.22	1.32*±0.12
VIII: Standard (Indomethacin)	1.50*±0.09	1.40*±0.04	1.44*±0.066	1.34*±0.22	1.28*±0.12

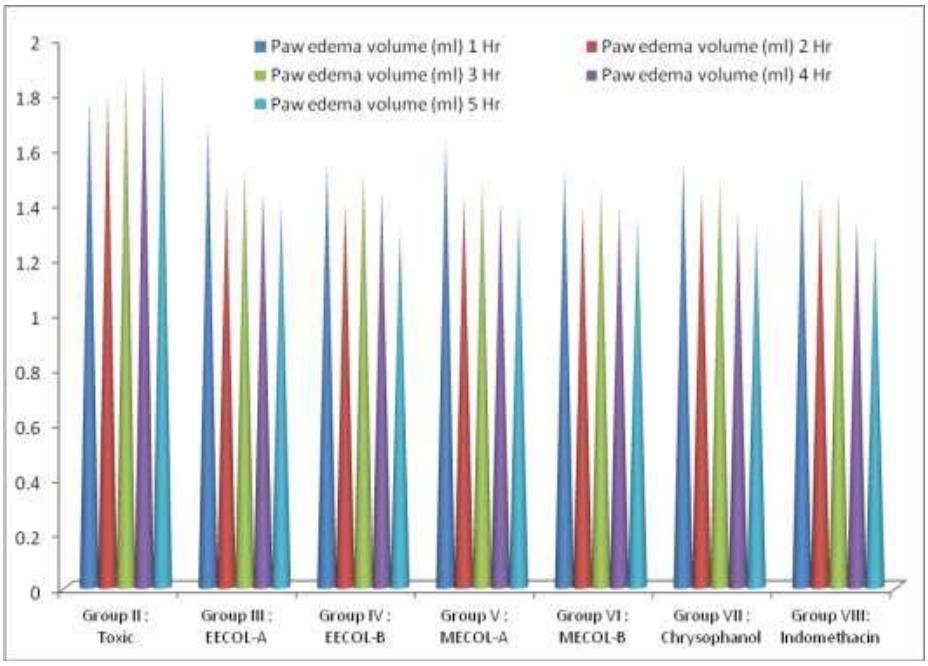

Figure 8: Effect of EECOL, MECOL and Chrysophanol on paw edema.

Table 9: Inhibition (%) of paw edema by EECOL, MECOL, and Chrysophanol.

Group	% of Inhibition of paw edema (1 to 5 hrs)				
	1	2	3	4	5
III : EECOL-A	5.74	16.48	18.99	22.58	25.92

IV : EECOL-B	10.34	15.93	20.6	21.5	31.22
V : MECOL-A	5.76	16.52	19.12	22.64	27.2
VI : MECOL-B	10.56	16.2	20.84	22.92	31.78
VII : Chrysophanol	11.24	16.6	20.2	23.4	32.4
VIII : Indomethacin	13.21	19.23	21.22	26.34	35.97

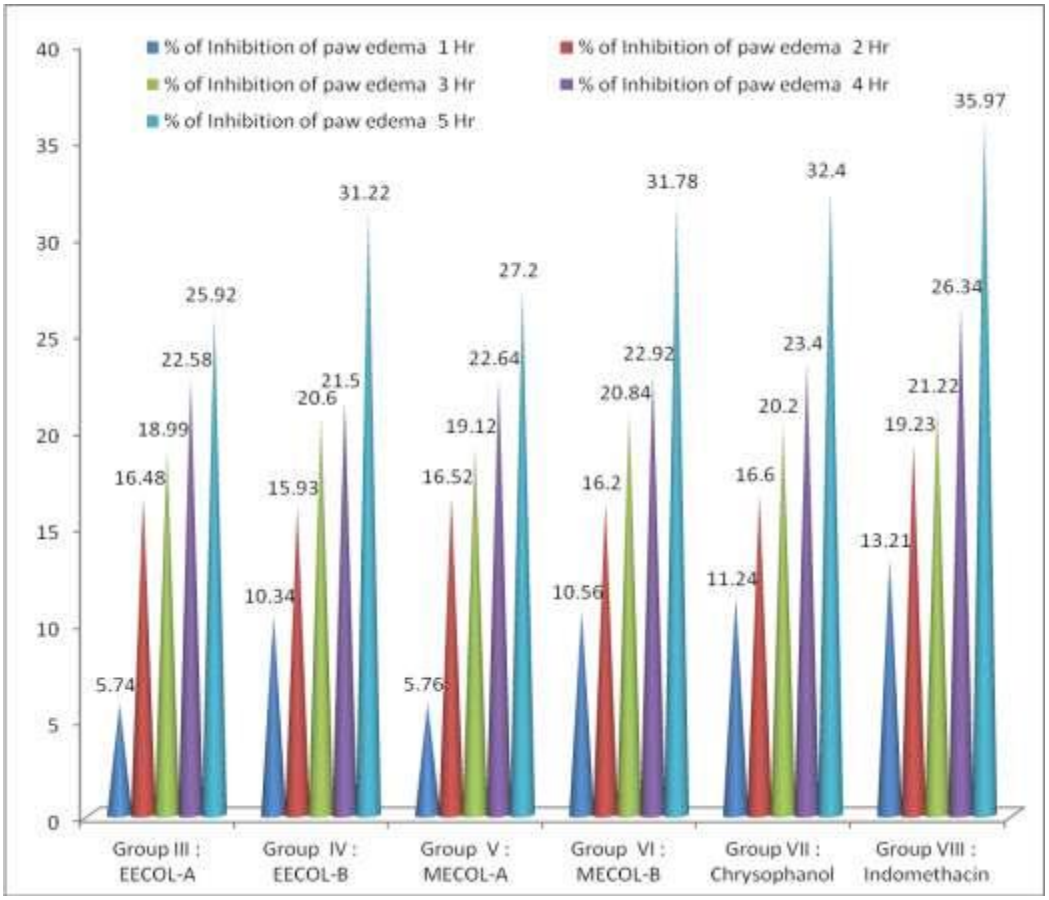

Figure 9: Effect of on inhibition (%) of paw edema in rats.

Wound Healing Properties of EECOL, MECOL Extractsand Chrysophanol

Wound healing studies (IAEC Form B approval: IEC/IAEC/2023/04 dated 17-02-2023) of EECOL, MECOL and Chrysophanol by excision wound method were carried out as method reported by Morton and Malone (1972) (Table 10).

Grouping of Animals and Procedure

Table 10: Grouping of Animals (6/group) for wound healing activity.

Group	Treatment and Dose
I :Normal Control	Vehicle control
II : EECOL-B	Drug Treated : EECOL-B (400 mg)
III : MECOL-B	Drug Treated : MECOL-B (400 mg)
IV : Chrysophanol	Chrysophanol (50 mg)
V : Soframycin	Soframycin Ointment (Standard)

(i) Animals were anaesthetized.
(ii) An excision wound (5cm; ring shaped) was made on dorsal thoracic region (depilated ethanol-sterilized) of male rats.
(iii) EECOL-B, MECOL-B and Chrysophanol were given to differentgroup of animals (group II-IV).
(iv) Subsequently, wound contraction was analysed by tracing the rawwound area on day 1, 4, 8, 12, 16, 18 and 21 using graph paper.
(v) To assess wound healing, parameters were estimated and recorded.

Results & Discussions

Cassia occidentalis L. (Kasondi) contains PPMs and SPMs of various chemical categories like anthroquinone, flavonoids, balsams, anthocyanosides, terpenes, alkaloids, saponins, cardiac glycosides, tannins, proteins, phlobatannins, steroids, phenolics, resins, amino acids, carbohydrates, and reducing sugars.

MECOL and EECOL were extracted by percolation methods and chrysophanol was isolated by IPTLC chromatographic method. Experimental practical yield of EECOL was 4.32% and practical yield of MECOL was found to be 3.8%. Isolated chrysophanol (practical yield - 10.6%) was found to be golden yellow crystalline powder with m.p.196^{0}C and UV λmax 277nm (poorly soluble in water and soluble in methanol).

Ash values indicated the inorganic salts or any extraneous matter (oxidation of the component of the crude drug; include carbonate, phosphates, silicates, and silica). Low ash value indicated drug was free from contamination. Low acid-insoluble ash values indicated that the drug was free from siliceous matters or sand. Water soluble ash value indicated presence of water soluble salts whereas sulphated ash showed oxides were converted into sulphates. Solvent extractive values depend upon the presence of phyto- constituents and useful for the determination of exhausted and adulterated drug (any addition of exhausted material to the pure drug will be reflected by lowering of these extractive values). LOD was performed to assess moisture content (percentage) as it cause enzyme hydrolysis, growth of microbes and leads to deterioration of PPMs and SPMs (Table 3-4).

EECOL, MECOL and chrysophanol proved good margin of safety (LD50of chrysophanol was 1000 mg/kg b.w and LD50 of MECOL and EECOL were 1600 mg/kg b.w; Table 5-7; Figure 6-7). At high dose, Chrysophanol MECOL, and EECOL Extract of Kasondi inhibited edema and produced significant anti- inflammatory effects. Edema inhibition was maximum with Indomethacin - standard drug (35.97%) followed by Chrysophanol (32.4%), MECOL-B (31.78%) and EECOL-B (31.22%) (Table 8-9; Figure 8-9). In wound healing activity, EECOL-B, MECOL-B extract and chrysophanol produced significant wound contraction with good epithelialization on day 15 and day 21. EECOL-B induced good wound contraction ($p>0.05$; 30.62% (4^{th}) → 44.24% (8^{th}) → 67.6% (12^{th}) and finally 87.6 % (15^{th}) (Table 10). Wound contraction of MECOL-B was also good ($p>0.05$; 29.22% (4^{th}) → 42.74% (8^{th}) → 65.20% (12^{th}) and 82.50% (15^{th}). Chrysophanol treated animals showed better wound contraction ($p>0.05$; 33 % (4) → 49.30% (8) → 72.56% (12) and 95.62% (15). Wound healing effect of EECOL, MECOL and Chrysophanol were comparable to soframycin ($p<0.01$; Table 10). Histology of EECOL, MECOL and Chrysophanol treated animals tissues showed good wound contraction with speedy epithelialisation, regeneration of new cells, high breaking strength with new blood vessel formation.

Conclusions

Research work was started with procurement of *Cassia occidentalis* L. and its scientific authentication. Percolation method was used for preparation of MECOL and EECOL extract and anthraquinone - chrysophanol was purified by IPTLC method. In toxicity assessment, no mortality was found and EECOL, MECOL and chrysophanol possess wide safety margin. In anti-inflammatory studies, chrysophanol MECOL, and EECOL Extract produced significant anti-inflammatory effects and edema inhibition was maximum with standard drug (Indomethacin; 35.97%) followed by Chrysophanol (32.4%), MECOL-B (31.78%) and EECOL-B (31.22%). In wound healing activity, treatment with MECOL-B, EECOL-B extract and chrysophanol produced significant wound healing (contraction with good epithelialization on day 15 and day 21). Wound healing effect of EECOL, MECOL and Chrysophanol were comparable to soframycin ($p<0.01$). Histology of MECOL, EECOL and Chrysophanol treated animals showed good would healing properties.

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