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Phytochemical Characterization, Antioxidant And Antihemorrhoidal Activities Of Hydroethanolic Extract Of Cleome Viscosa L.

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Abstract:
Background*: Hemorrhoids and liver disorders related to oxidative stress are common health problems. Existing treatments are only partially effective and can sometimes be toxic. Cleome viscosa L., rich in secondary metabolites, is traditionally used for its anti-inflammatory and hepatoprotective effects. The objective of this study was to evaluate the antioxidant, anti-inflammatory, and hepatoprotective activities of the hydroalcoholic extract of Cleome viscosa in rats with induced hemorrhoids, comparing two doses (250 and 500 mg/kg) to Daflon at 10 mg/kg*
Materials and Methods*: The methodology involved preparing aqueous-ethanolic extracts of Cleome viscosa L., followed by phytochemical screening tests, and administering two doses (250 and 500 mg/kg) to rats after experimental induction of hemorrhoids. The parameters studied included serum protein levels, liver enzymes (ALT, AST), the recto-anal coefficient, and antioxidant activity (DPPH assay).*
Results*: The extract contained 54.7 mg QE/g of flavonoids and 289.6 mg GAE/g of polyphenols, in addition to other qualitatively identified secondary metabolites. The DPPH IC was 0.287 mg/mL, confirming notable antioxidant activity. The erythrocyte sedimentation rate decreased from 3.33 to 1.67 with C. viscosa L. at 250 mg/kg, while serum proteins increased from 52.03 to 62.47 g/L. At this dose, the recto-anal coefficient dropped to 0.00287, AST decreased to 206.43 U/L, and ALT to 65.48 U/L, indicating significant improvement in inflammation and liver protection. At 500 mg/kg, the effects were moderate, suggesting an optimal dose of 250 mg/kg for extract efficacy ($p<0.05$).*
Conclusion: *Overall, Cleome viscosa demonstrates high therapeutic potential due to its antioxidant, anti-inflammatory, and hepatoprotective properties, confirming its relevance as a safe natural alternative in the management of hemorrhoids and liver disorders.*
Key Word: *Cleome viscosa, secondary metabolites, antioxidant, anti-inflammatory, hemorrhoids*

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I. Introduction

Local inflammatory diseases such as hemorrhoids are associated with intense inflammatory responses, alterations in hematological parameters, and systemic oxidative stress, requiring safe and effective treatments[1,2]. Conventional anti-inflammatory drugs often present gastrointestinal and hepatic side effects that limit their long-term use, thus motivating the search for natural phytotherapeutic alternatives with a good safety profile[3,4]. Several medicinal plants rich in flavonoids and polyphenols have shown remarkable anti-inflammatory and antioxidant effects, which could help attenuate both local inflammation and systemic oxidative stress[5,6,7,8,9,10,11].

Cleome viscosa L., a medicinal plant widely used in India, China, Africa, and other tropical regions, is traditionally employed to treat various inflammatory disorders as well as gastrointestinal conditions and

wounds[12]. Studies have shown that flavonoid glycosides isolated from *C. viscosa* significantly reduce experimentally induced inflammation, while its extract exhibits substantial antioxidant activity, attributed to the polyphenols and flavonoids present in the plant[13]. Other research has highlighted hepatoprotective, analgesic, and antimicrobial properties of this species, suggesting a versatile pharmacological profile relevant to complex inflammatory conditions[14,15].

However, despite these bioactive properties, few studies have investigated the use of *Cleome viscosa* in the specific context of hemorrhoids, which involve combined local vascular disturbances, inflammation, and oxidative stress.

The objective of this study is therefore to evaluate the antioxidant, anti-inflammatory, and hepatoprotective efficacy of the hydroalcoholic extract of *Cleome viscosa* in rats with induced hemorrhoids.

II. Material And Methods

Materials

Vegetal Material: *Cleome viscosa L.* plants were collected early in the morning around the University Center of Natitingou. The entire plant material (leaves, stems, roots, and seeds) was used, as the local population reported its use in this form.

Chemical Materials : The chemical materials included methanol, ethanol, hydrochloric acid, gallic acid, and quercetin, supplied by Sigma-Aldrich and Acros Organics. Various types of laboratory glassware were also used throughout the experiment.

Animal Material : The animal material consisted of twenty-five (25) Wistar albino rats with an average body weight of 130 g. These rats were randomly divided into five (5) groups of five rats each and housed in standard cages for a two-week acclimatization period prior to experimentation. They were maintained at a constant temperature of 22 ± 2°C and subjected to a 12-hour light/12-hour dark cycle in the animal facility of the Research Unit in Applied Microbiology and Pharmacology of Natural Substances, University of Abomey-Calavi.

Methods

Powder Preparation : *Cleome viscosa L.* plants were carefully washed and then air-dried at room temperature for 7 days [16]. The dried plants were then finely ground using an electric grinder. The resulting powder was sieved and stored in glass containers protected from moisture until further use.

Preparation of extracts: Sixty grams (60 g) of *Cleome viscosa* L. powder were mixed with 600 mL of a water/ethanol (96%) solution in a 30 :70 ratios, with the water preheated to 100°C. This method has previously demonstrated efficiency in optimizing the extraction of bioactive compounds[17]. After agitation and homogenization, the mixture was filtered using Whatman filter paper. The filtrate was concentrated using a rotary evaporator at 45–50°C under reduced pressure with the aid of a vacuum pump. Final drying was carried out in an oven at 35°C for 72 hours. The dry extract obtained was stored at 4°C in a refrigerator.

Qualitative phytochemical screening of hydroethanolic extract of *C. viscosa* : Qualitative phytochemical screening was performed based on colorimetric and precipitation reactions. The tests were carried out directly on the hydroethanolic extract of *C. viscosa* L. powder according to the method described by Houghton and Raman (1998)[18].

Quantitative phytochemical screening of hydroethanolic extract of *C. viscosa* L.: Quantitative phytochemical analyses were performed according to the method of Harborne (1984), adapted to our laboratory conditions[19,20].

Determination of total polyphenols: Total polyphenol content was determined using the Folin–Ciocalteu colorimetric method. This reagent is reduced during the oxidation of phenolic compounds into a mixture of blue oxides of tungsten and molybdenum. The resulting blue coloration shows maximum absorbance around 750 nm. The absorbance, compared to a standard calibration curve obtained with gallic acid, allowed the quantification of total phenolic content expressed as mg gallic acid equivalents per gram of extract (mg GAE/g)[20].

Determination of total flavonoids: Total flavonoid content was estimated using the aluminum chloride (AlCl) method. Quercetin was used as the reference compound for establishing the calibration curve[20].

Polyphenol and flavonoid contents were calculated using the following formula:

$T = (C \times Vr) / (Vp \times Cp)$

Where: T = content of compounds; C = concentration obtained from the calibration curve; Vr = reaction volume; Vp = volume of extract used; Cp = concentration of the extract solution

***In vitro* evaluation of antiradical activity:** The antiradical activity of the extracts was evaluated using the DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, following a procedure similar to that described by Fagbohoun (2014)[20]. This method is based on the reduction of the stable free radical DPPH• in the presence of a hydrogen-donating compound at a wavelength of 517 nm. The percentage of DPPH radical scavenging was calculated using the following formula:

% DPPH = 100 × (Abs blank – Abs sample) / Abs blank

Where: Abs blank = absorbance of the control (DPPH solution); Abs sample = absorbance of the test sample;

The IC value, defined as the concentration of extract required to neutralize 50% of DPPH radicals, was determined from the graph plotting percentage inhibition against extract concentration. Each test was performed in duplicate[21].

Evaluation of biological parameters

Grouping of animals

Table no 1 presents the grouping of rats, the substances administered, and the corresponding doses used. Each group consisted of five (5) rats.

Table no 1: Composition of groups

Groups	Codes	Substances to be administered
Group 1 Normal Control	**GN1**	No induction and treatment with distilled water
Group 2 Negative Control	**GN2**	Induction without treatment
Group 3 Reference Control	**RCD 10 mg/kg**	Induction and treatment with Daflon at 10 mg/kg
Group 4	**CV 250 mg/kg**	Induction and treatment with NB at 250 mg/kg
Group 5	**CV 500 mg/kg**	Induction and treatment with NB at 500 mg/kg

CV = hydro-ethanolique extract of *Cleome viscosa* L.

Induction of hemorrhoids: Hemorrhoids were induced in rats using a 6% croton oil solution prepared by mixing distilled water, pyridine, acetone, and croton oil in a ratio of 1:4:5:10. Prior to induction, the rats were subjected to overnight fasting.

Induction was performed in all groups except the normal control by inserting sterile cotton swabs (4 mm in diameter), soaked with 100 µL of the 6% croton oil solution, into the anus (recto-anal region, approximately 20 mm from the anal opening). This procedure was carried out once daily for three consecutive days[22].

On day 4, blood samples were collected to measure erythrocyte sedimentation rate and total protein levels. Photographs of the animals were also taken.

Anti-hemorrhoidal test and measurement of biological parameters: On the fourth day, each rat in the test groups received the corresponding treatment dose (Table 1) orally for 5 days. The animals were weighed and anesthetized using ether. A 1 cm of the rectum was surgically excised from each rat. The rectal tissues were weighed and analyzed to evaluate weight variations between control and treated groups[23]. Photographs of rats from the different groups were also taken.

Recto-anal coefficient (RAC): The recto-anal coefficient (RAC) was calculated using the following equation:
RAC = Recto-anal tissue weight (mg) / Body weight (g)

Inflammatory index: The inflammatory index was assessed on day 9. To evaluate the severity of inflammation in the recto-anal region, a scoring system based on clinical hemorrhoidal presentation and severity index was used. Hemorrhoids were graded on a scale from I to IV: **Grade I:** Anal cushions bleed but do not prolapse; **Grade II:** Anal cushions prolapse during straining but reduce spontaneously; **Grade III:** Anal cushions prolapse during straining and require manual reduction; **Grade IV:** Prolapsed tissue remains permanently outside and is irreducible[23].

Biochemical parameters: On day 9, blood samples were collected from all animals to measure erythrocyte sedimentation rate, total protein levels, aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT), in accordance with OECD guidelines (2025)[24].

Data processing and analysis: The collected data were entered into Microsoft Excel 2013 and subjected to appropriate statistical analyses. All results were expressed as mean ± standard deviation. Analysis of variance (ANOVA), followed by Tukey's multiple comparison test, was used to compare percentages and variation rates. The level of statistical significance was set at 5% ($p < 0.05$).

III. Result

Yield and Physical Appearance of the Extract

Table no2 presents the characteristics of the *Cleome viscosa* L. (CV) extract obtained using a water/ethanol mixture (30/70). This extract is distinguished by a pasty physical appearance, indicating a semi-solid texture after extraction. The extraction yield is 12.36 ± 1.83%. The code assigned to this extract is "CV," an abbreviation of the plant's scientific name, facilitating its identification in the study.

Table no2: Physical Appearance and Codes

Extracts	Physical appearance	Extraction yield (%)	Codes
Cleome viscosa L. water/ethanol (30/70)	Paste	12,36 ± 1,83	**CV**

Phytochemical Composition of composition of *Cleome viscosa L.*

Table no3 presents the phytochemical composition of *Cleome viscosa L.* (CV), highlighting a diversity of metabolites. The extract contains reducing compounds, alkaloids, flavonoids (54.7 mg QE/g), and a high content of total polyphenols (289.6 mg GAE/g). Catechic and gallic tannins, anthocyanins, leuco-anthocyanins, terpenoids, and mucilages are also present. In contrast, saponins, carotenoids, quinonic compounds, free anthracenic compounds, and O-heterosides are absent.

Table no 3: Phytochemical composition of *Cleome viscosa L.* (CV)

Compounds	CV	Compounds	CV
Reducing compound	+	Saponin	-
Alkaloids	+	Terpenoids	+
Flavonoids (mgEQ/g)	54,7	Mucilages	+
Tanins catechic	+	Cartenoids	-
Tanins gallic	+	Free Anthracenics	-
Anthocyanins	+	O-heterosides	-
Leuco-anthocyanins	+	Polyphenols totaux (mgEAG/g)	289,6
Quinonics compound	-		

Antioxidant Activity by the DPPH Assay

The results for *Cleome viscosa* show an initial DPPH value of 0.960, with a target value of y = 0.480 corresponding to 50% inhibition. After solving the equation, the IC is estimated at 2.87×10^{-1} mg/mL, i.e., 0.287 mg/mL. This value indicates a notable antioxidant activity of the extract, reflecting its ability to neutralize free radicals, although it remains moderate compared to more potent reference standards.

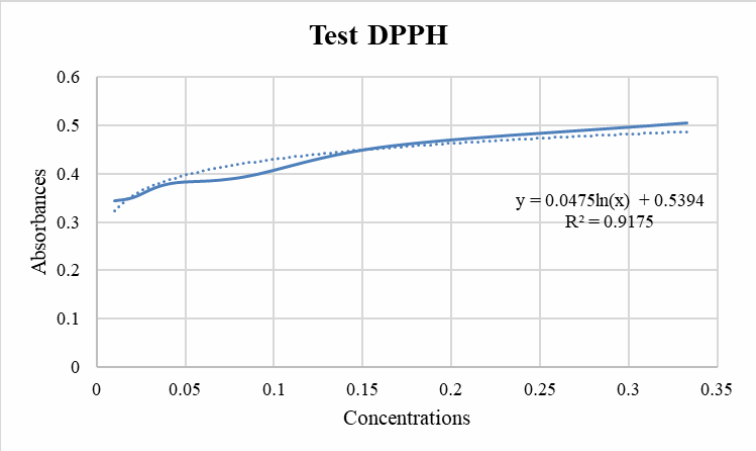


Figure no1: Antioxidant activity by the DPPH assay

Evaluation of anti-hemorrhoidal activity

Distribution of Experimental Groups

The table no1 in the Materials and Methods section shows the distribution of experimental groups and the treatments administered to the rats. Group 1 (GN1) served as the normal control, without induction or treatment, receiving only distilled water. Group 2 (GN2) was the negative control, subjected to induction without treatment. Group 3 (RCD 10 mg/kg) received Daflon after induction. Groups 4 (CV 250 mg/kg) and 5 (CV 500 mg/kg) were treated with *Cleome viscosa* extract at doses of 250 mg/kg and 500 mg/kg, respectively, after induction. This setup allows for a comparative evaluation of treatment effectiveness. The experimental design is relevant, as it enables a clear comparison between no treatment, standard treatment, and the tested extract, facilitating interpretation of potential therapeutic effects.

Effect of Treatments on Erythrocyte Sedimentation Rate in Rats after Hemorrhoid Induction

Figure no2 presents changes in the erythrocyte sedimentation rate (ESR) in rats following hemorrhoid induction and treatment. The normal control group remained stable, with values of 2 before and after treatment. The negative control decreased from 2.67 to 2, while the group treated with Daflon at 10 mg/kg increased slightly, from 2.67 to 3. The *Cleome viscosa* extract at 250 mg/kg significantly reduced the ESR from 3.33 to 1.67 ($p < 0.05$), indicating a notable anti-inflammatory effect, whereas the 500 mg/kg dose increased slightly from 4 to 4.33, suggesting a less favorable effect at the higher dose.

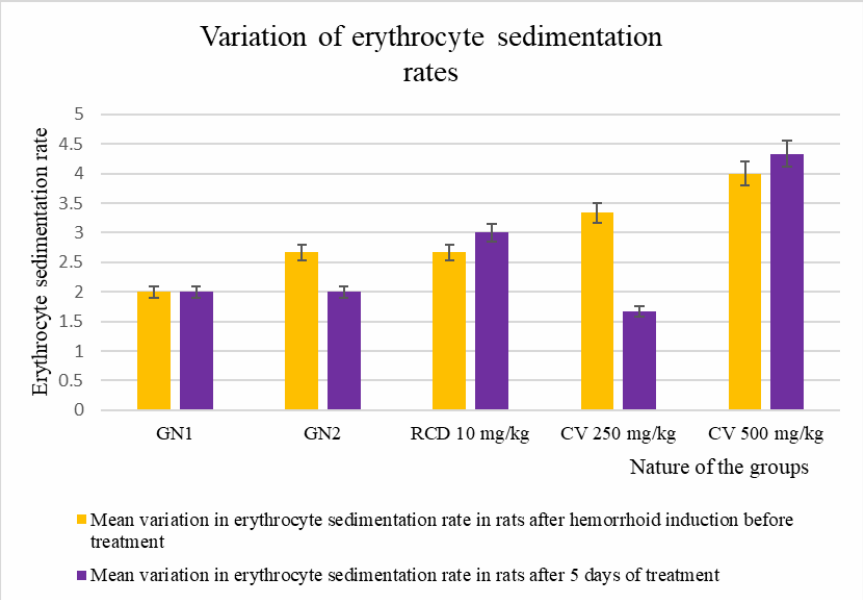


Figure no2: Erythrocyte sedimentation rate in rats after hemorrhoid induction

Effect of the extract on total protein levels in rats after hemorrhoid induction and treatment

Figure no3 presents the mean serum protein levels in rats following hemorrhoid induction and treatment. The normal control group (GN1) showed a slight increase from 58.07 to 62.4 g/L ($p < 0.05$). The negative control group (GN2) decreased from 57.5 to 48.5 g/L, reflecting the pathological effect. Daflon at 10 mg/kg increased slightly from 61.77 to 62.15 g/L, indicating stabilization. The *Cleome viscosa* extract at 250 mg/kg produced a significant increase from 52.03 to 62.47 g/L ($p < 0.05$), suggesting a restorative effect. At 500 mg/kg, the extract slightly decreased from 62.87 to 60.5 g/L, confirming a moderate effect at the higher dose.

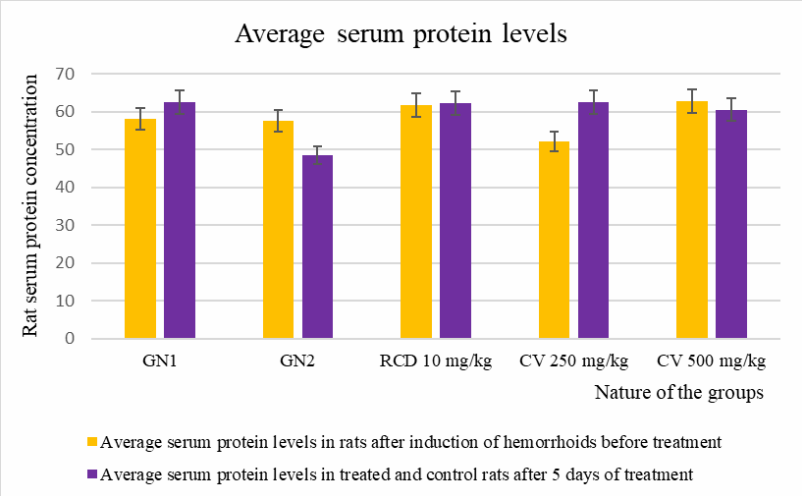


Figure no3: Total protein levels in rats after hemorrhoid induction and treatment

Effect of the extracts on the variation of the recto-anal coefficient in rats after hemorrhoid induction

Figure no4 shows the average variations of the recto-anal coefficient (RAC) after treatment with different extracts. The normal control group (GN1) displayed an RAC of 0.00335, whereas the negative control (GN2) showed a higher value of 0.00510, indicating functional impairment ($p < 0.05$). The reference group treated with Daflon (10 mg/kg) had an RAC close to normal, 0.00346. *Cleome viscosa* extract at 250 mg/kg reduced the RAC to 0.00287, suggesting a significant improvement, while the 500 mg/kg dose gave 0.00331, still indicating a moderate effect.

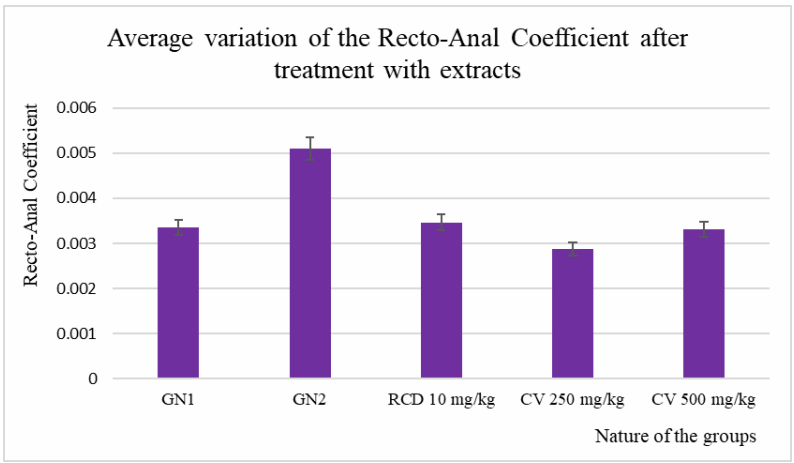


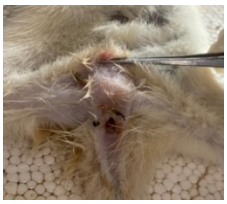
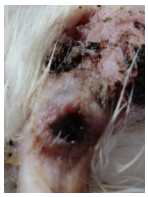
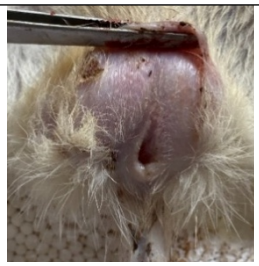
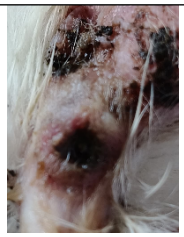
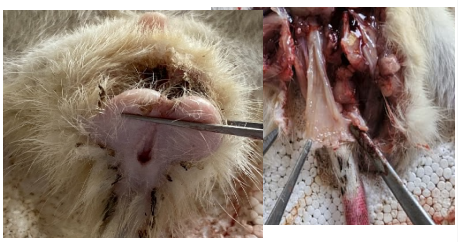
Figure no4: Variation of the recto-anal coefficient in rats after hemorrhoid induction

Inflammatory Index

The results show that the treated groups (RCD 10 mg/kg and *Cleome viscosa* at 250 and 500 mg/kg) exhibited a notable improvement in symptoms, progressing from grade II to grade I, characterized by the absence of prolapse. This regression indicates a potential therapeutic effect, likely related to the anti-inflammatory and vasoprotective properties of the plant extracts. The lack of improvement in the control groups confirms the efficacy of the treatments. Furthermore, the comparable effect between the two doses suggests that the extract is active even at lower concentrations.

Table no4 : Clinical evaluation of hemorrhoids after 5 days of treatment according to experimental groups

Groups	Before treatment	After treatment	Appreciation after treatment
GN1			

			
GN2			Grade II: The anal cushions prolapse through the anus during straining but reduce spontaneously
RCD 10 mg/kg			Grade I: The anal cushions bleed but do not prolapse
CV 250 mg/kg			Grade I: The anal cushions bleed but do not prolapse
CV 500 mg/kg			Grade I: The anal cushions bleed but do not prolapse

Effect of Extracts on Serum ASAT Levels in Rats After Hemorrhoid Induction and Treatment

Figure no5 shows the average ASAT levels in rats after five days of treatment. The normal control group (GN1) displayed 325.22 U/L, while the negative control (GN2) showed a significant increase to 438.98 U/L, reflecting hepatic stress. The group treated with Daflon (10 mg/kg) had 369.98 U/L, close to normal. Cleome viscosa extract at 250 mg/kg markedly reduced ASAT to 206.43 U/L, indicating a significant protective effect ($p < 0.05$), whereas the 500 mg/kg dose maintained a moderate level of 345.07 U/L, suggesting a dose-dependent efficacy.

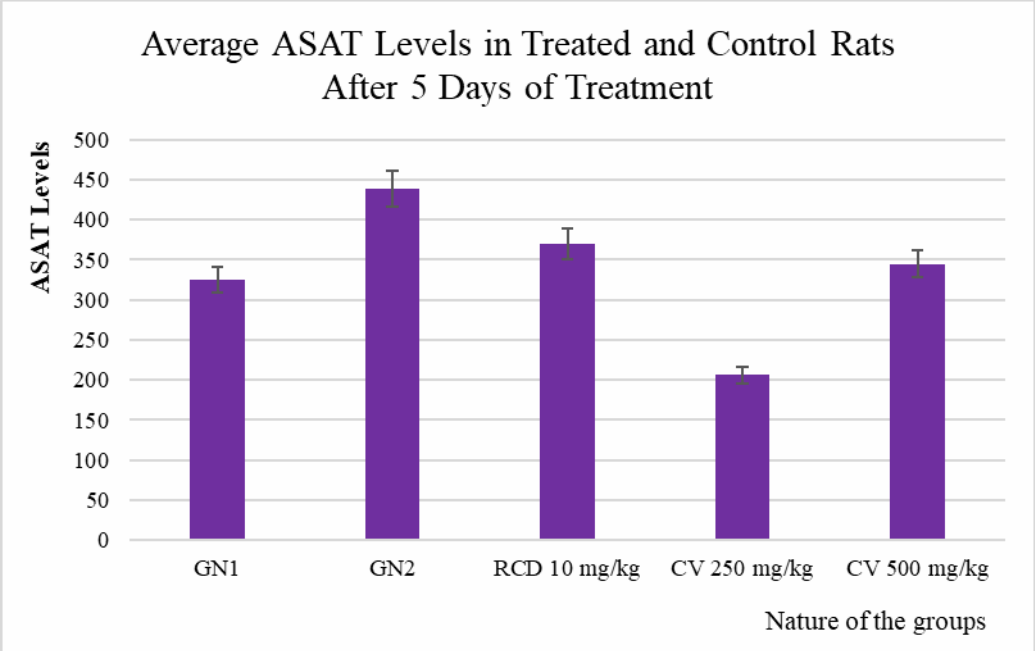


Figure no5 : ASAT Levels in Rats After Hemorrhoid Induction and Treatment

Effect of Extracts on Serum ALAT Levels in Rats After Hemorrhoid Induction and Treatment

Figure no6 shows the average ALAT levels in rats after five days of treatment. The normal control group (GN1) had a low value of 50.22 U/L, indicating normal liver function. The negative control (GN2) showed a significant increase to 118.47 U/L, indicating hepatic damage. Daflon at 10 mg/kg showed a surprisingly high value of 180.36 U/L for a reference treatment. Cleome viscosa extract at 250 mg/kg reduced ALAT to 65.48 U/L, suggesting effective hepatoprotection, while the 500 mg/kg dose reached 89.51 U/L, indicating a moderate effect.

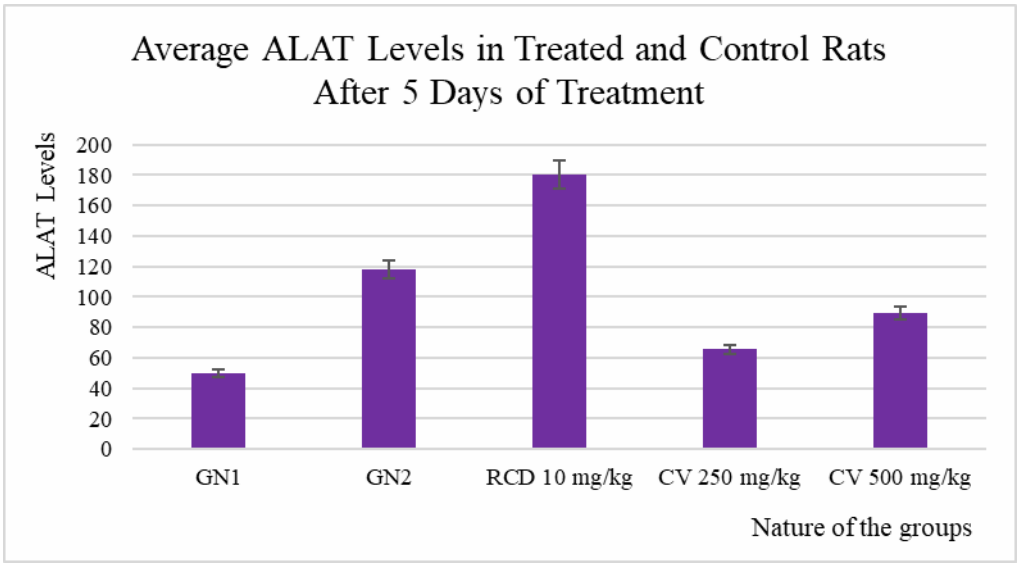


Figure no6 : ALAT Levels in Rats After Hemorrhoid Induction and Treatment

IV. Discussion

This study highlights that the hydroalcoholic extract of *Cleome viscosa* L. (CV) possesses significant phytochemical diversity, with high levels of flavonoids (54.7 mg QE/g) and total polyphenols (289.6 mg GAE/g) (Table no3). These classes of metabolites are well known for their antioxidant and anti-inflammatory activities, as demonstrated by the extract's ability to scavenge free radicals in the DPPH assay, with an IC of 0.287 mg/mL (figure no1). These findings are consistent with previous studies on this species: Senthamilselvi et al. (2012) and other authors have shown that various molecules isolated from *C. viscosa* exhibit significant *in vivo* anti-inflammatory activity through inhibition of induced edema and free radical scavenging [25,26,27].

At the hematological and biochemical levels, treatment of hemorrhoid-induced rats with *C. viscosa* at 250 mg/kg was associated with a significant improvement in serum protein concentrations, suggesting restoration of physiological status disrupted by inflammation (figure no2) ($p < 0.05$). This effect is likely related to the reduction of tissue and metabolic damage through the combined antioxidant and anti-inflammatory action of the bioactive compounds[28]. Indeed, recovery of serum proteins is a recognized marker of resolution of systemic inflammation and cellular damage[29]. Similarly, studies on polyphenol-rich extracts indicate that these compounds can protect plasma proteins from oxidative stress-induced damage, thereby reducing protein loss or denaturation associated with inflammation[30,31].

Regarding liver function, administration of CV at 250 mg/kg markedly decreased hepatic injury enzymes (ASAT, ALAT), suggesting hepatoprotective effects. These results align with previous studies showing that *C. viscosa* extracts reduce serum transaminases and attenuate histological alterations in the liver in CCl_4 induced hepatotoxicity models, comparable to the protective effects of standard agents such as silymarin[14,32].

The reduction in the recto-anal coefficient (RAC) observed with *C. viscosa* further supports a functional anti-inflammatory effect, likely mediated by inhibition of the pro-inflammatory mediator cascade through flavonoids, polyphenols, and other metabolite classes present (figure no4). This finding has not previously been reported for this plant.

Overall, the data converge toward a versatile pharmacological profile for *Cleome viscosa*, characterized by antioxidant, anti-inflammatory, hepatoprotective effects, and potential modulation of hematological parameters. This justifies its successful exploration as a phytotherapeutic agent in complex inflammatory conditions such as hemorrhoids.

V. Conclusion

This study demonstrates that the hydroalcoholic extract of *Cleome viscosa*, rich in secondary metabolites, possesses significant antioxidant, anti-inflammatory, and hepatoprotective properties. The 250 mg/kg dose appears optimal, improving erythrocyte sedimentation rate, serum proteins, and recto-anal coefficient, while reducing hepatic enzymes (ASAT, ALAT). These results confirm the therapeutic potential of *Cleome viscosa* as an effective natural alternative for managing hemorrhoids and protecting liver function.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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