

Synergistic Anti-Acne Activity of Bombax ceiba Thorn and Curcuma longa Gel in Testosterone-Induced Acne Vulgaris in Rodents

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Research Article

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Abstract

Purpose

The present study purpose was to study and analyses the anti-acne activity of bombax ceiba thorn and curcuma longa combination against testosterone induced acne vulgaris on rodent.

Methods

Curcuma longa and Bombax ceiba thorn were gathered. to ascertain the bombax ceiba's Pharmacognostical assessment (phytochemical screening, microscopic estimation, physical evaluation, and macroscopic/organoleptic) to use GCMS analysis to assess Bombax cebia's phytoconstituents and in vitro antioxidant activities to create a gel-based formulation of bombax ceiba extracts with curcuma longa and assess the formulation's assessment parameters. To assess the anti-inflammatory and anti-acne properties of a gel-based formulation and a combination extract of bombax cebia and curcuma longa using an in vivo model of testosterone-induced acne to assess inflammatory markers like IL-1 β and TNF- α . to assess, using histopathological analysis, the decrease in the number of sebaceous glands in the skin of mice.

Result

Treatment with EEBC + CL (**P < 0.05) the level of IL-1 β TNF- α , MDA levels, oxidative stress, lipid peroxidation, reduction of sebaceous gland number, reduction of papules, inflammation reduced

Conclusion

The gel formulation with extracts of Bombax ceiba and Curcuma longa may be useful in treating testosterone-induced acne vulgaris, according to the study's findings. It improved antioxidant defence while dramatically lowering oxidative stress, TNF- α , IL-1 β , and acne severity. Its safety and effectiveness need to be confirmed by additional clinical trials.

Introduction

The eighth most common illness in the world is a chronic inflammatory disease that affects people of all ages is acne vulgaris. (Madeleine et al. 2017). A few skin eruptions, including pustules, papules, cysts, blackheads, and whiteheads, are indicative of acne vulgaris. When it comes to acne vulgaris, lesions can be either non-inflammatory or inflammatory, or both. (Chilicka et al. 2020). Acne-causing factors include the following: More sebum is produced by SG, along with hypercornification of the sebaceous ducts, Propionibacterium acnes colonization of the pilosebaceous ducts, and inflammation (Evans 2009). The common commensal bacterial species (Chaudhary et al. 2012). Redness, scarring, or hyperpigmentation

can arise from *Propionibacterium* inflammation brought on by acne and inflammatory lesions, such as pustules, nodules, and papules infected in the dermis around the comedone or microcomedone (Benner et al. 2013), (Gollnick et al. 1991). The prevalence of acne vulgaris is highest among teenagers, accounting for 9.4% of the global population. In all ethnic groupings, it affects more than 90% of men and 80% of women (Yosipovitch et al. 2007),(Gandolfi et al. 2015) Known as acne vulgaris, it is skin that is eighth most prevalent ailment globally, with a frequency of 9.38% calculated for all age groups. According to the Global Burden of Disease Study 2010 (Vos et al. 2012). Topical retinoids are versatile agents in the treatment of acne. For noninflammatory acne, topical retinoids are recommended as a monotherapy; for inflammatory acne, they should be used in conjunction with antibiotics (Sharma2017). The FDA in the United States has authorized Azelex, a cream formulation, to treat acne vulgaris; however, Finacea, a gel, has a far higher bioavailability (Kadhim et al. 2016). The most fundamental and important component of traditional medicine is the medicinal plant. Herbal medicines are gaining popularity due to their benefits, which include increased tolerance, a track record of extended use, fewer side effects, and a reduced cost. Various plants, usually referred to as herbal plants, contain medicinal qualities. The Bombacaceae family plant *Bombax ceiba* L has a wide range of pharmacological activities(Strauss et al. 2007).This work deal with to Ameliorate effect of ethanolic extract and extract-based gel formulation of *Bombax ceiba* thorn and *Curcuma longa* combination against testosterone induced acne vulgaris in rodent. This plant has different phytoconstituents which shows anti-inflammatory, antioxidant, antibacterial, antimicrobial activity etc.

Material and method

Collection and identification of plant

The *Bombax ceiba* thorn were collected from jawala ji, Himachal pradesh, India. The plant material was identified and authenticated by Mr. R.S Jayasomu chief scientist Head, RHMD, CSIR-NIScPR in New Delhi, India, verified the plant as "*Bombax ceiba*" thorn.

Extraction of bombax ceiba

After the tree's thorns were removed, it was dried for a specified amount of time in the shade, and then ground into a powder. A continuous hot Soxhlet extraction was performed on the dried powder (100 g, split into several smaller portions) over 32 cycles at a temperature of 55–65°C, using an equal ratio of 50 milliliters of alcohol or distilled water (ethanol 90%). A rotating vacuum evaporator was used to extract the solvent at a regulated temperature and lowered pressure. *Bombax ceiba* (TEBC) hydroalcoholic thorn extract A yield of 11.8% w/w was found. (Evans et al. 2017)

Extraction of curcuma longa

Curcuma longa and Zingiber officinale rhizomes were gathered, chopped into tiny pieces, and allowed to air dry at 35–400 degrees Celsius for two weeks. Using a Soxhlet device and 70% ethanol (a hydroalcoholic solvent), extraction was carried out. For future research, the extracts were dried, condensed under low pressure, and kept in airtight containers at 40 degrees Celsius.

Antioxidant test in vitro

Antioxidant assay for DPPH

In figure.1 (a) and (b) shown DPPH presence is shown by a pink color in both standard and sample tubes. After adding DPPH, radicals are stabilized, changing the color from pink to light pink. The DPPH free radical is an organic nitrogen radical that has a distinct deep purple color. When an antioxidant is introduced to a DPPH solution, a reaction occurs, causing the color to change from purple to colourless, indicating the formation of the corresponding hydrazine compound. Antioxidants' ability to lower DPPH can be evaluated by observing a drop in absorbance between 515 and 528 nm. This reduction is indicative of the antioxidant's effectiveness in scavenging DPPH. The results are typically presented as IC₅₀ (the concentration required to impede half of DPPH) or as the percentage of DPPH scavenged by a fixed concentration of the antioxidant across all samples Brand-Williams et al. 1995. Figure 2 has been shown below IC₅₀ of standard and sample in µg/ml.

Formulation of Gel

Figure 3 has shown different formulation of *Bombax Cebia* and *Curcuma longa* combination. Every component was gathered in the prescribed quantity. In two separate beakers, the formulation elements were mixed for this. Water was split evenly between two beakers. The appropriate amount of plant extracts was added to the first beaker and allowed to dissolve. Then, a determined quantity of propylene glycol, followed by the addition of Carbopol 940 in a second beaker dissolved, and EDTA and methyl paraben were added. Subsequently, the two beaker solutions were combined into a single beaker, and finally, drop by drop, triethanolamine was added to achieve the desired coherence of the developed formulation. Various Parameter assessed during gel formlation of *Bombax ceiba* thorn and *Curcuma longa* combination shown in Table 1.

Assessment of the gel formulation that was developed

(Pawar et al. 2013)

The following parameters were used to evaluate the manufactured gel formulations:

Assessment of PH

The pH of the produced gel compositions was measured using a pH meter.

Appearance and homogeneity

The physical characteristics and uniformity of the produced formulations were examined visually.

Viscosity

It was measured at 100 rpm using a Brookfield viscometer equipped with spindle number 6.

Spreadability

The diameter of one gram of gel spread between two glass-covered slides was used to measure it. Spreadability was established.

The formula for calculating Spreadability was $S = M \times L / T$.

Where, S stands for Spreadability, M for pan weight (attached to the top slide).

L is the length that the glass slide moved.

T is the amount of time (in seconds) needed to fully separate the slides.

In vivo model for the acne vulgaris

Experimental Animals

20–30g was the average body weight of male Swiss albino mice, will purchase from Akal college of pharmacy and technical education, mastuana sahib sangrur, Punjab and stored in the animal house of laureate Institute of pharmacy, kathog, H.P. The animals was house in polyurethane cage in well-regulated environment with a room temperature of 25 to 20 degrees Celsius and a relative humidity of 60%. The animal was fed a commercial meal consisting of pellets and unlimited water. Every behavioural measure was evaluated from 9:00 to 17:00. With the animal approval number, the Institutional Animal Ethics Committee (IAEC) authorized the study's protocol was CCSEA/LIPH/2020/30 and follow the rules for the purpose of using and caring for test animals set out by the Indian National Science Academy (INSA). To prevent intergroup conflict, age-matched animals will be used in all studies for the specified treatment.

Hematological parameters

Serum total protein

IGF-1 and the hormone testosterone are both produced in greater quantities, which contribute to acne.

Triglycerides

The severity of acne was correlated with a gradual derangement in the lipid profile, notably in the triglycerides.

Cholesterol

Unhealthy blood cholesterol levels begin to accumulate beneath the skin and may cause acne.

Pro-Inflammatory marker for TNF- α , IL-1 β includes levels of TNF- α , IL-1 β .

Estimation of MDA level

The following were added: A 20% acetic acid solution in 1.5 milliliters (pH 3.5, corrected with sodium hydroxide), 200 μ L Ten percent w/v tissue homogenate, 1.5 mL of thiobarbituric acid (0.8%) and 200 microliters of aqueous sodium dodecyl sulphate (8.1%). After adding distilled water to provide the total amount to 4 mL, the mixture was heated for one hour at 95°C in a water bath. After cooling the liquid, 1 milliliter of distilled water and 5 milliliters of a 15:1 n-butanol and pyridine were combined and added. For five minutes, it was centrifuged at 5000 rpm. After removing the top layer, absorbance at 532 nm was measured utilizing a spectrophotometer both visible and UV. (Ohkawa et al.,)

Estimation of Nitrite level

Using a colorimetric assay, the concentration of nitrite, a sign of the production of (NO), in the striatum supernatant was based on the 0.1% N-(1-naphthyl). Greiss reagent (ethylenediamine dihydrochloride, 1% sulphanilamide, and 2.5% phosphoric acid). The spectrophotometer utilized was a Shimadzu to detect absorbance at 540 nm. The standard curve was used to calculate the amount of nitrite in the supernatant (Singh *et al.* 2015).

Histopathology

After collecting blood samples, the animals were euthanized using cervical dislocation. The skin tissue was extracted from the animals. Assessments were made of morphological alterations (lesion count, hyperkeratinization of sebaceous glands).

Results and discussion

Plant authentication

Mr.R.S Jayasomu chief scientist Head, RHMD,CSIR-NIScPR in New Delhi,India, verified the plant as '*Bombax ceiba*' thorn .The specimen received an authentication certificate with number NIScPR/RHMD/Consult/2023/4512-13, and the certificate is enclosed in the annexure.

Formulation of gel

After being manufactured, the herbal gel was evaluated according to a number of factors. 2%, 4%, 6%, and 8% extracts were used in the development of many formulations; however, the third formulation, which included 6% extract, had more efficacy than the other preparations. When applied, it felt smooth and chilly and had a brownish tint and transparent aspect. Ph remained consistent throughout the

investigation, and it was discovered that the gel did not cause skin irritation when applied. Measurements of Spreadability revealed that it was less variable than the gel that had been previously created. The gel did not irritate the skin when it was applied.

Table 1 Various Parameter assessed during gel formlation of *Bombax ceiba* thorn and Curcuma longa combination

Formulations	Ph	Apperarance	Viscosity	Spreadability	Homogeneity
1	6.46	Yellowish- brown	4455	43	well
2	6.47	Yellowish brown	4476	42	well
3	6.49	Brownish	4478	44	Excellent
4	6.51	Brownish	4479	41	well

Evaluation of In-vivo Anti-acne activity

Total protein

On the fourteenth day, the group that had acne developed had higher levels of total protein than the control group. In comparison to the acne-induced groups, the level was lower in all treated groups following a 28-day course of therapy with the appropriate medicine.

Triglycerides

In the acne-induced group, triglyceride levels were lower on day 14 than in the control group. In comparison to groups that had acne, all treated groups had higher levels following a 28-day course of therapy with the appropriate medication.

Cholesterol

On the fourteenth day, the group with acne had higher cholesterol than the control group. In comparison to the acne-induced groups, the level was lower in all treated groups following a 28-day course of therapy with the appropriate medication. Haematological markers in groups with acne before and after therapy has been shown below inTable 2.

Table 2
Haematological markers in groups with acne before and after therapy

Parameter	Result	Control	Disease	Treatment 1	Treatment 2	Standard
Total protein(g/dl)	Pre	5.02 ± 0.29	5.92 ± 0.14	5.39 ± 0.12	5.36 ± 0.12	5.30 ± 0.15
	Post	5.10 ± 0.10	6.40 ± 0.50	5.96 ± 0.12	5.90 ± 0.12	5.99 ± 0.35
TGA(mg/dl)	Pre	65.5 ± 2.10	140.5 ± 3.50	71.5 ± 1.13	71.0 ± 1.12	91.0 ± 5.66
	Post	68.5 ± 3.52	138.5 ± 3.50	135.0 ± 2.10	133.5 ± 2.10	138.5 ± 4.9
Cholestrol	Pre	56.5 ± 3.52	93.5 ± 3.52	92.0 ± 2.8	90.0 ± 2.8	93.0 ± 2.8
	Post	55.0 ± 2.80	97.0 ± 1.40	60.0 ± 2.10	58.0 ± 2.8	79.5 ± 2.12

Estimation of inflammatory mediators

Effect of EEBC combination curcuma longa and formulation on skin tissue TNF- α against testosterone induce acne vulgaris in mice

Estimation of inflammatory mediators

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In Figure.4 Biochemical parameters TNF- α , estimation. All groups subjected to TNF- α biochemical estimation. The level of TNF- α was significantly increase in disease groups as compare to control groups ($p < 0.001$). The treatment group are significantly decreasing the amount of TNF- α as compare to disease group ($p < 0.05$).

Effect of EEBC combination curcuma longa and formulation on skin tissue IL-1 β against testosterone induce acne vulgaris in mice

In figure.5 All groups subjected to IL-1 β biochemical estimation. The level of IL-1 β was significantly increase in disease groups as compare to control groups ($p < 0.001$). The treatment with EEBC + CL and gel formulation significantly decrease the amount of IL-1 β as compare to disease group ($P < 0.05$).

Effect of the extracts and the gel formulation on sample tissue MDA level against testosterone induced acne model in mice.

The level of MDA significantly increase in disease group as compared to control group ($p < 0.001$) after tratment with EEBC + CL and gel formulation significantly decrease the level of MDA ($p < 0.005$) in Fig. 6.

Effect of the extracts and the gel formulation in the in vivo-anti acne testosterone induced model in mice in nitrite level

All groups subjected to nitrate biochemical estimation. In figure.7 the level of nitrate are significantly increase in disease groups as compare to control groups ($p < 0.001$). The treatment group EEBC + CL and topical formulation significantly decrease the amount of nitrate as compare to disease group ($p < 0.05$).

Estimation of skin tissue parametrs

Chloroform anaesthesia was used to put the mice to sleep, and tissue samples were taken from their skin and placed and using 10% buffered formalin saline for histology. Following the cutting of the paraffin-embedded tissues into slices that were 4 to 5 microns thick they were stained for standard histopathology using hematoxylin and eosin stain (H&E). Routine light microscopy was used to evaluate morphological alterations (lesion count, sebaceous gland hyperkeratinization). After 28 days of therapy with the appropriate medications, the Histology Lesion Count was graded in each group mentioned in Table 3.

Table 3

After 28 days of therapy with the appropriate medications, the Histology Lesion Count was graded in each group.

Sr.no	Groups	Observatory event			
		Inflammation	Blockage of sebaceous glands	Hyperkeratinization	Total number of lesions
1	Regular saline	–	–	–	–
2	Diseases	++++	++++	++++	++++
3	Treatment 1	++	++	++	++
4	Treatment 2	+	+	+	+
5	Standard		+++		–

Refer to the lesion count grading index.

Extremely elevated +++

Elevated +

Typical

+Low

Definitely absent

Cross observatory changes in acne induced mice mode

Cross observation shows normal dorsal side of skin in control group only given the normal saline. In disease group-2 dorsal side of skin gets inflamed with papules, blackheads, whiteheads, nodules, pustules. Group 3 treatment 1 and group 4 treatment 2 shown reduction of papules as compare to standard.

Figure 8 Group I (A) histological observation (Control; normal saline treatment) and Group II (Disease Control) histological alterations (B)

Histological observation in Group I (Control; normal saline treated) there is no inflammation and papules but sebaceous glands were found intact in shown Fig. 8A Histological observation in group II Disease group the microscopic examination of tissue sections of disease group acne lesions and inflammatory cells. Sebaceous gland showed clogging and presence of papules in large amount.

Figure 8 Histological changes in Group III treatment of extracts BC + CL Fig D Histological changes in Group IV formulation of BC + CL

Histological observation in group III examination of tissue sections of skin sample and applied B.C + C. L treated acne and determined lesions revealed less clear and reduced less level of inflammatory cells shown in shown in Fig C. In group IV examination of tissue section of skin and applied topical gel formulation of combination B.C and C.L treated acne and determined lessions revealed clear and reduced level of inflammatory cells and reduction of papules was shown in Fig. 8E.

Discussion

The skin eruptions of acne vulgaris include whiteheads, black heads, pustules, papules, and cysts. Inflammatory, noninflammatory, or a combination of both types of lesions can be seen in acne vulgaris. The medicinal plant is the most basic and significant element of our healthcare system. Herbal plants are used to make most medications. The Bombaceae family Group III histological alterations Group IV 71 contains the silk cotton tree, *Bombax ceiba* (Malvaceae), which has histological alterations. The solvent extraction technique was used in the prior work to isolate certain secondary metabolites from *B. ceiba* L. flowers in excellent yield, and their anti-diabetic effectiveness was assessed in vitro. The current research of *Bombax ceiba* thorn extract is another that is used to treat mouth ulcers. Fruit has Leprosy-friendly, cooling, digestible, diuretic, antioxidant, tonic, aphrodisiac, expectorant, and exhibits favorable genital urinary organ activity. Additionally, it is utilized as a blood purifier, kidney, bladder, and chronic inflammation treatment. Pile therapy involves the application of root bark. The root has astringent and diuretic properties. It helps with inflammation, biliousness, and excessive body heat. The combination of *bombax ceiba* and *curcuma longa* has good antiacne action since both plants exhibit anti-inflammatory properties, according to a detailed analysis of acne vulgaris from a literature survey. Nowadays, the

majority of people are using herbal therapies that have few negative effects since they are worried about the negative consequences of synthetic pharmaceutical medications. Bombax ceiba thorns were gathered from Jawala Ji in India's Himachal Pradesh. The thorns were removed from the bark, allowed to dry in the shade for a predetermined amount of time, and then ground into a suitable powder. Ethanol was used as a solvent in a continuous Soxhlet device test on the dried powder. and another is curcuma longa that has been dried for a certain amount of time and appropriately powdered. Ethanol, a solvent from the Soxhlet equipment, was continuously applied to the dried powder. Several phytochemical parameters determined by GCMS estimation. The different phytoconstituents found in extract to treat illness were identified by these experiments. both qualitative and quantitative analysis of the extract's different phytoconstituents. Flavonoid content estimate, tannin estimation, and phenol estimation were all quantitatively determined. As a result, every phytochemical examination showed that the ethanolic extract we examined contained more flavonoids than phenolic or tannin. Alkaloids, flavonoids, diterpenes, phenols, and the lack of triterpenes were all qualitatively analysed. Bombax ceiba thorn's phytochemical characteristics include a brownish hue, a conical shape, a rough texture of 17–25 mm, and a bitter taste. The phytochemical parameters revealed that the distilled water extract had the highest extractive value, measuring 29.18%, when compared to the acid insoluble ash insoluble extractive value.

This could be because the bombax ceiba thorn plant that was chosen has a high concentration of polar chemicals. A drying value loss of 11.6%w/w was discovered. Because of its high moisture content, the powder had a high initial weight before drying. The powder's water content evaporates when heated in a hot air oven, resulting in a drop in the powder's ultimate weight of 72 after drying. Important indicators that show the quality and purity of the crude medicine as well as the presence of inorganic material in it are the total ash and acid-insoluble ash levels. The quality of the thorn cannot be determined only by the amount of ash present. Therefore, another metric to show the quality of medications is the amount of acid-insoluble ash. It was discovered that the acid-insoluble value was 29.15% and the total ash value was 29.26%. The percentage of total ash was higher than that of acid-insoluble value. A formulation for topical gel was created. Aziderm gel was used as the standard, and other formulations including 2%, 4%, 6%, and 8% extracts of bombax ceiba and a mixture of curcuma longa were made. Based on the gel formulations' anti-acne activity results, it was determined that the gel containing 6% extract of the formulation's total quantity exhibited the best activity out of all the preparations. Before the in vivo trial began, all pharmacognostical or formulation preparations were made. Each group consists of five male Swiss albino mice, weighing between 20 and 30 g. To induce acne in 14 days, 2% testosterone was administered. After 14 days, see whether there is a decrease in inflammation or papules. Combinational extracts of B.C. and C.L. were administered from day 15 to day 28 of treatment 1. Additionally, treatment 2 was administered using the produced gel formulation. The skin of the sacrificed animal was removed, and several biochemical or histological characteristics were measured. Biochemical characteristics IL-1 β and TNF- α estimate. Every group underwent biochemical measurement of TNF- α . TNF- α levels are much higher in disease groups (274.33 ± 27.10) than in control groups. TNF- α levels are much lower in the therapy group than in the illness group. Furthermore, compared to the other treatment groups, treatment 2 exhibits a greater drop in TNF- α (165.167 ± 1.179). Every group underwent biochemical

measurement of IL-1 β . When compared to control groups, the illness groups' levels of IL-1 β are considerably higher (206 ± 1.179). When compared to the illness group, the therapy group's levels of IL-1 β are much lower. Furthermore, as compared to the other treatment groups, treatment 2 exhibits a greater decrease in IL-1 β (126.167 ± 2.357). All groups undergoing nitrite biochemical estimation will have their antioxidant activity evaluated. The illness groups had a considerably higher nitrate level (199.044 ± 0.615) than the control groups. TNF- α levels are much lower in the therapy group than in the illness group. In addition, treatment 2 exhibits a greater decrease in TNF- α (118.60 ± 0.615) than the other treatment group. Papules, an increase in the sebaceous gland, lesions, and inflammation were among the histological criteria to be evaluated in the disease group. The highest results were shown in treatment 2 with fewer papules, a smaller sebaceous gland, and less irritation.

Conclusion

To sum up, acne vulgaris is a long-term inflammatory disorder that can impact individuals of all ages. Acne increases sebum production from the pilosebaceous units when it affects highly visible areas like the face, chest, and back regions with dense sebaceous follicles. Follicle hyperkeratinization and clogging of the hair follicle follow, which encourages the growth of anaerobic microorganisms like *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) in the clogged follicle. Heat, swelling, redness, and pus are the results of the skin being irritated due to these microbes. These phytochemicals are useful in the treatment of acne vulgaris because they have been shown to have anti-inflammatory, antioxidant, and antibacterial qualities. Overall, the project's findings show that the gel formulation with extracts of *Bombax ceiba* and *Curcuma longa* may be useful in treating testosterone-induced acne vulgaris. Numerous acne-related metrics were improved when the herbal gel composition was administered. Compared to control groups, illness groups had considerably higher levels of TNF- α and IL-1 β . When compared to illness groups, the level of IL-1 β and TNF- α is dramatically reduced by the EEBC + CL and gel formulation therapy. A decrease in MDA levels signifies a decrease in oxidative stress and lipid peroxidation, but an increase in catalase activity points to a stronger defense against free radicals. Additionally, the histological study showed that the treated mice had significantly fewer sebaceous glands, papules, and lesion numbers. This suggests that the EEBC + gel formulation successfully lessened the severity of skin imperfections and acne lesions. To confirm these results and investigate the formulation's safety and effectiveness for wider uses in acne treatment, more research is necessary, including clinical trials involving human participants.

Declarations

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Competing Interest. The authors have no relevant financial or non-financial interests to disclose.

Author Contribution.

The authors confirm contribution to the paper as

- **Kiran Bala:** Conceptualization, Methodology, Investigation, Data Curation, Writing – Original Draft Preparation, Writing – Review & Editing.
- **Sidhant Sharma:** Investigation, Writing – Review & Editing, Validation, Visualization.
- **Vandana Bhatia:** Supervision, Formal Analysis.
- **Shiv Kumar Kushawaha:** Project Administration.
- **Shad Ahmed:** Formal Analysis.

All authors have read and approved the final manuscript. The authors confirm that no paper mill and AI was used.

Data Availability

The data supporting the findings of this study are available within the article and its supplementary materials. Due to ethical considerations and the proprietary nature of the formulations used, the raw data and detailed experimental protocols cannot be publicly shared.

Ethics Approval

This study was conducted in accordance with the ethical standards of [Laureate institute of pharmacy kathog] and the Declaration of Helsinki. The research protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Laureate Institute of Pharmacy, under registration number CCSEA/LIPH/2020/30.

Clinical Trial Number

Not Applicable.

Consent for Publication

This study was approved by the Institutional Animal Ethics Committee (IAEC) of L.R Group of Institute, Registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India CCSEA/LIPH/2020/30. The research adhered to the 'Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998' and followed the principles of the 3Rs—Replacement, Reduction, and Refinement—to ensure ethical treatment of animals.

Human Ethics and Consent to Participate

Not Applicable.

Consent to Publish

This manuscript does not involve human participants; therefore, specific consent for publication is not applicable.

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Figures



Figure 1

(a) In both the standard and sample tubes, the presence of DPPH is indicated by Pinkish color (b) After adding DPPH to both standard and sample tubes, the DPPH radicals are stabilized, leading to a change in the solutions' color from pink to light pink.

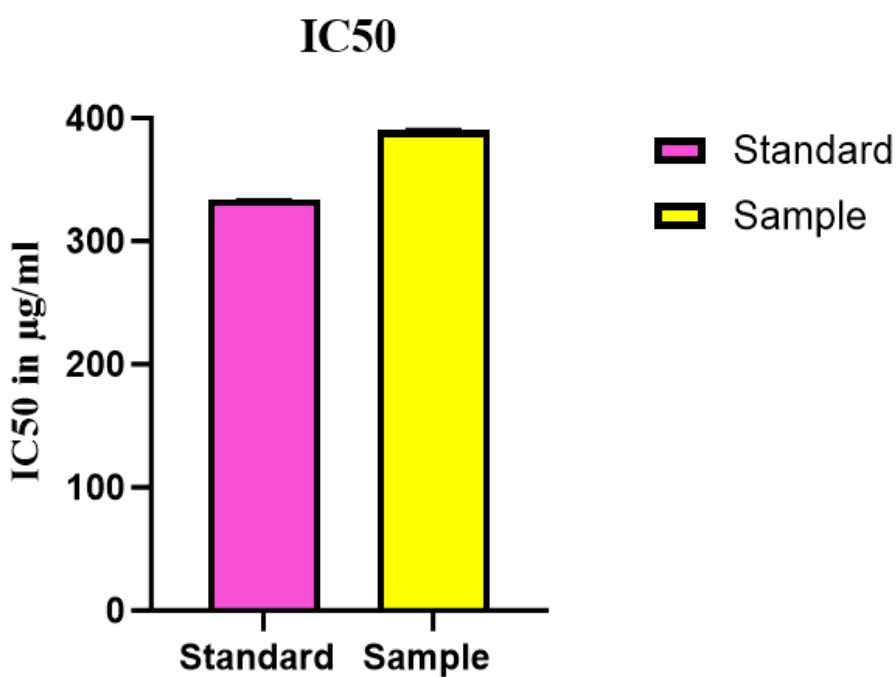


Figure 2

The IC50 of standard and sample in $\mu\text{g/ml}$

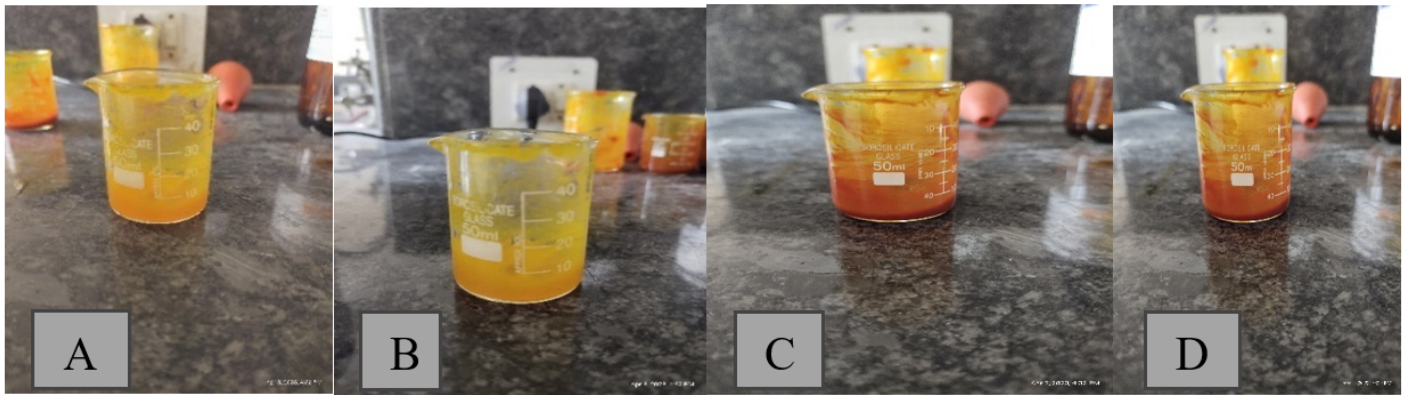


Figure 3

Different formulation of *Bombax Cebia* and *Curcuma longa* combination

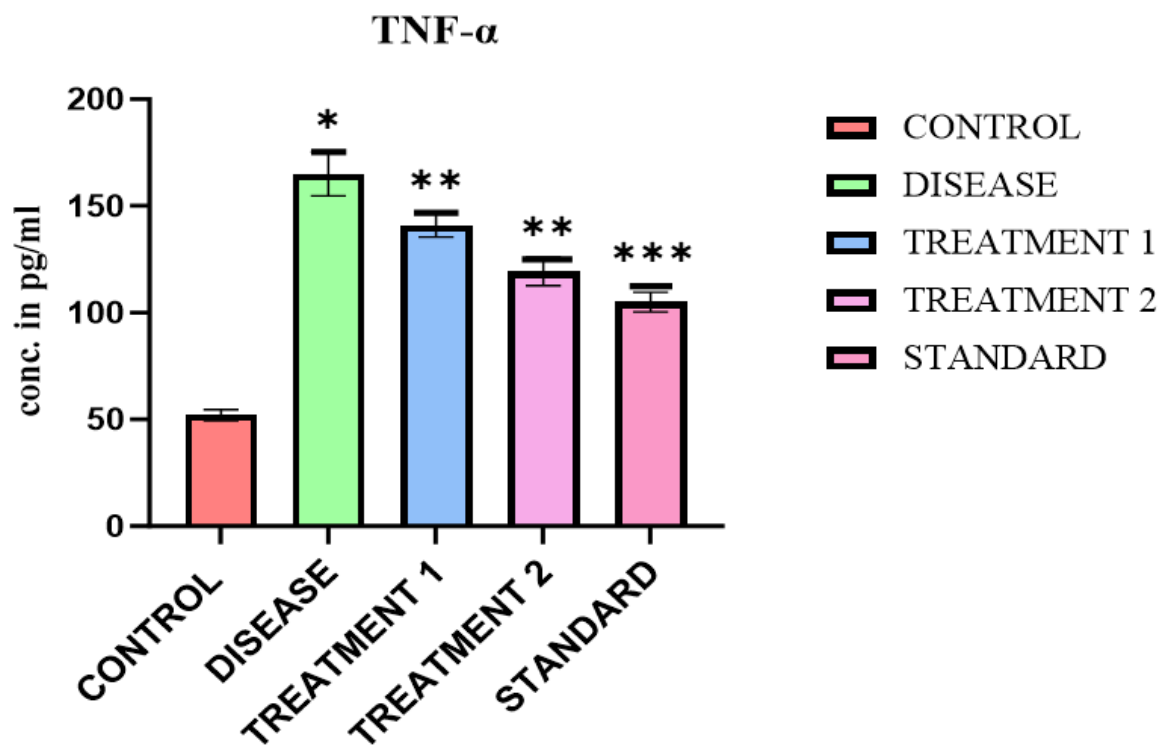


Figure 4

Effect of EEBC or curcuma longa combination and formulation on skin tissue TNF- α againsts testosterone induce acne model in mice. The data are investigation using one way Anova followed by Dunnett's

multiple comparison test value expressed as mean $\pm$ SD (N=5) a $P<0.001$ Vs Control,b $P<0.05$ Vs testosterone,c $P<0.001$ Vs testosterone.

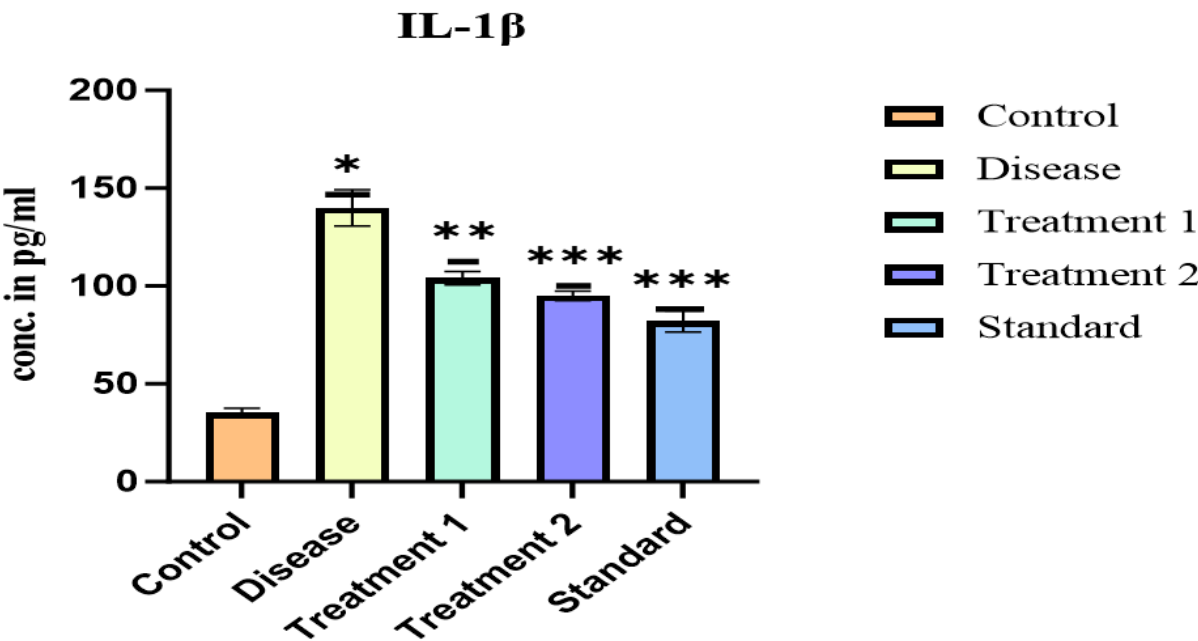


Figure 5

Effect of EEBC or *curcuma longa* combination and formulation on skin tissue IL-1 β against testosterone induce acne model in mice.The data are investigation using one way Annova followed by Dunnett’s multiple comparison test value expressed as mean $\pm$ SD (N=5) ^a $P<0.001$ aVs Control,^b $P<0.05$ Vs testosterone,^c $P<0.001$ Vs testosterone.

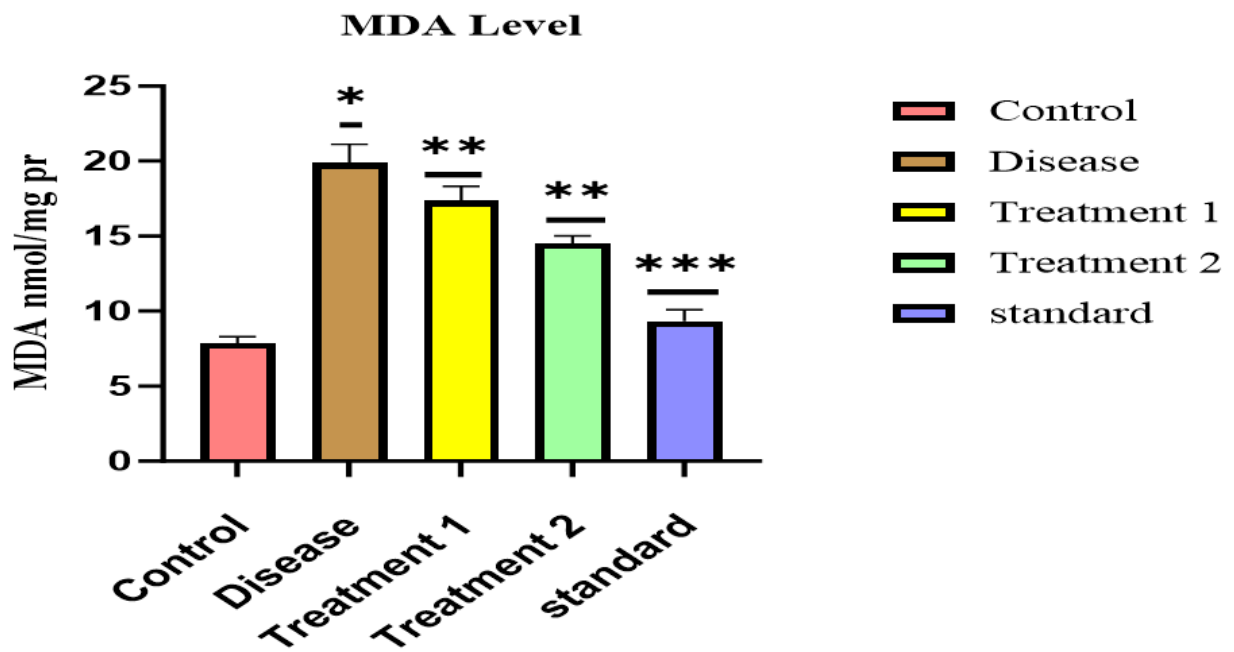


Figure 6

Effect of EEBC or *curcuma longa* combination and formulation on skin tissue MDA level against testosterone induce acne model in mice. The data are investigation using one way Anova followed by Dunnett's multiple comparison test value expressed as mean $\pm$ SD (N=5) ^a $P < 0.001$ Vs Control, ^b $P < 0.05$ Vs testosterone, ^c $P < 0.001$ Vs testosterone.

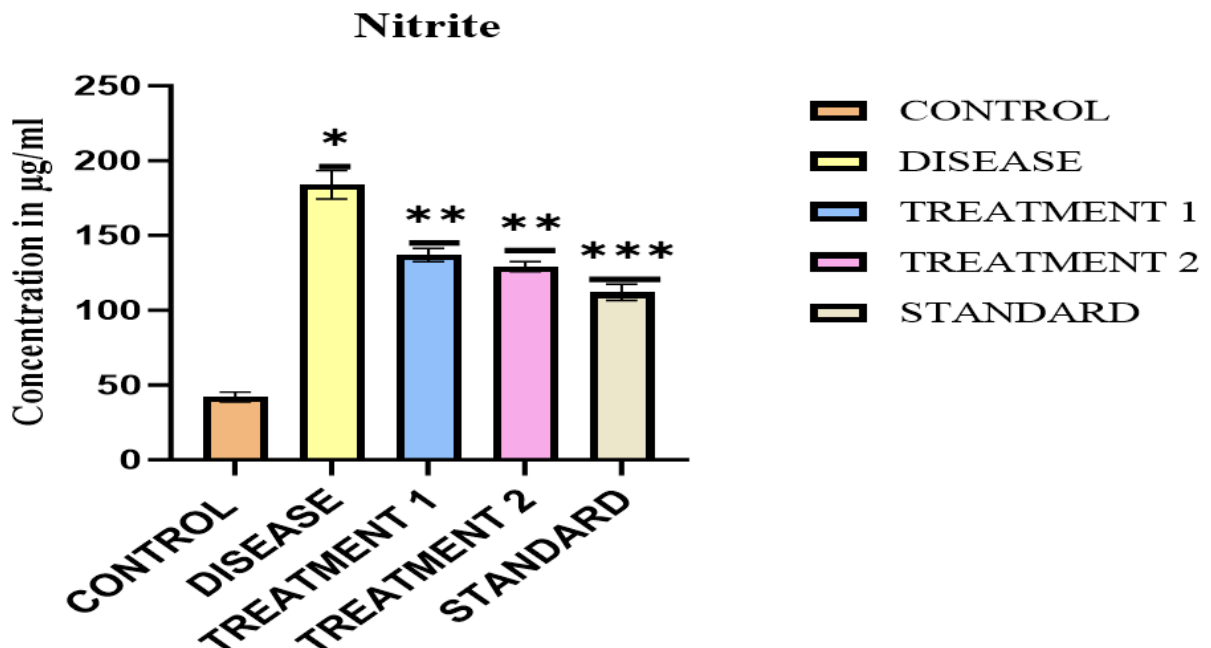


Figure 7

Effect of EEBCand formulation on skin tissue Nitrite level against testosterone induced acne in mice. The data are investigated using one way Anova folloed by Dunnett's multiple comparison test value expressed as mean $\pm$ SD (n=5). *P<0.001 Control Vs **P<0.05 Vs Disease, ***p<0.05 Vs Disease, ***p<0.001 Vs Disease

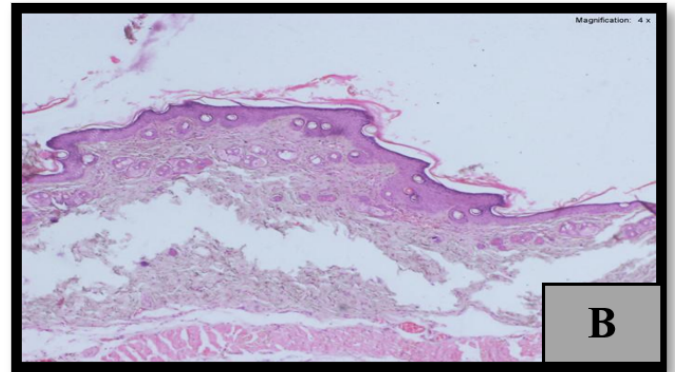
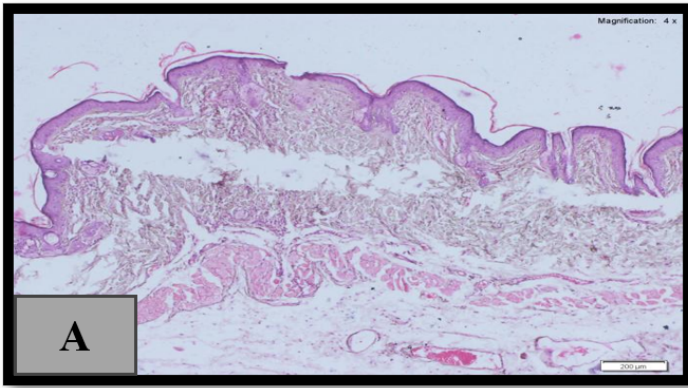


Figure 8

Fig.8 Group I (A) histological observation (Control; normal saline treatment) and Group II (Disease Control) histological alterations (B)

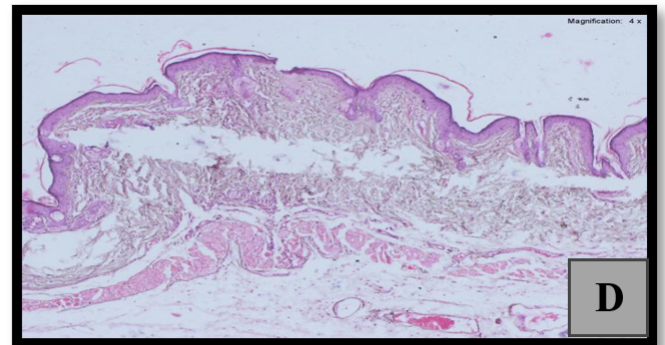
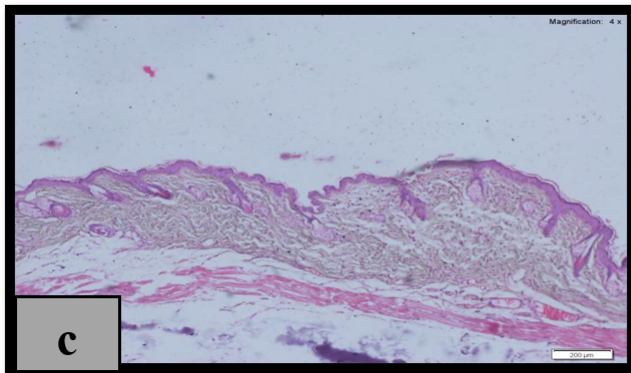


Figure 9

Fig.8 Histological changes in Group III treatment of extracts BC+ CL Fig D Histological changes in Group IV formulation of BC+ CL

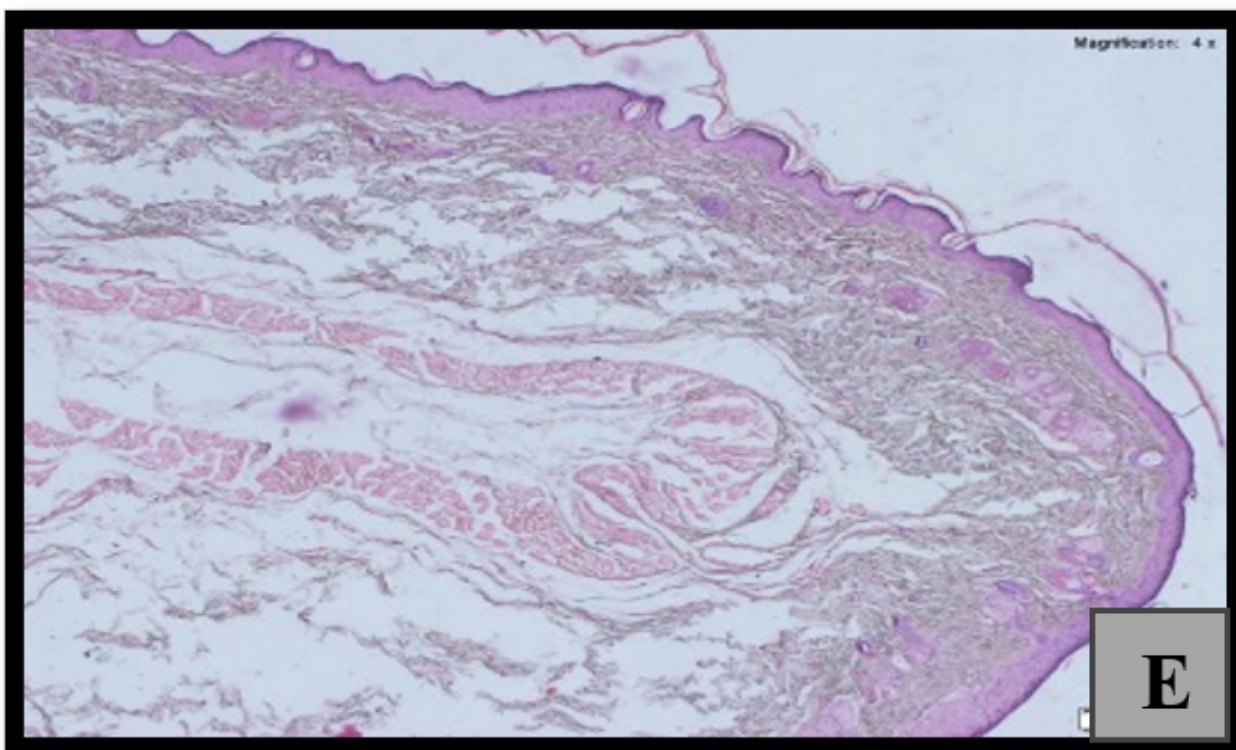


Figure 10

Fig.8 In group 5 examination of tissue section of skin and applied topical Standard Aziderm formulation more reduction.