

EVALUATION OF ORAL ADMINISTRATION OF POLYHERBAL LEAF EXTRACT OF *ANNUNA SQUAMOSA* AND *OCIMUM SANCTUM* ON THE ANTI-INFLAMMATORY ACTIVITIES INDUCED IN RATS

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ABSTRACT

The present study investigated the anti-inflammatory potential of a polyherbal formulation prepared from the leaves of *Annonasquamosa* and *Ocimum sanctum*, collected locally from Jodhpur, Rajasthan, India, and authenticated by the Botanical Survey of India. Anti-inflammatory activity was evaluated using carrageenan- and egg albumin-induced rat paw edema models, tissue punch weights, cytokine profiling, and histopathological examination. Carrageenan and egg albumin significantly increased paw volume, tissue weight, and pro-inflammatory cytokines (IL-6, TNF- α), while suppressing the anti-inflammatory cytokine IL-10, confirming induction of acute inflammation. Diclofenac sodium (Group III) served as a positive control, effectively reducing edema, tissue weight, and restoring cytokine balance. Among the polyherbal formulations, the 50:50 ratio of ASME:OSWE (Group V) demonstrated the greatest efficacy, significantly attenuating paw edema, reducing tissue weight, downregulating IL-6 and TNF- α , and restoring IL-10 levels, with effects comparable to diclofenac. The 25:75 (Group IV) and 75:25 (Group VI) ratios showed moderate activity, underscoring the importance of extract proportion in determining therapeutic potential. Histopathological evaluation further confirmed that the 50:50 formulation reduced edema and epithelial hyperplasia more effectively than other ratios. Overall, the findings suggest that the balanced 50:50 ASME:OSWE formulation exerts synergistic anti-inflammatory effects and represents a promising natural alternative to conventional therapy. Future studies should focus on isolating active phytoconstituents and elucidating the molecular mechanisms underlying this synergism.

KEYWORDS: *Annona squamosa*, *Ocimum sanctum*, Polyherbal, Anti-inflammatory, Cytokines, Carrageenan and Egg Albumin

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Conflict of interest: None.

INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants¹. Although it is a protective mechanism, persistent or uncontrolled inflammation contributes to the progression of several chronic diseases, including arthritis, cardiovascular disorders, and metabolic syndromes². Conventional anti-inflammatory drugs, while effective, are often associated with adverse effect which has prompted increasing interest in herbal and polyherbal formulations as safer alternatives³.

Annonasquamosa (commonly known as custard apple) is a tropical plant belonging to the family Annonaceae⁴. Traditionally, its leaves have been used in folk medicine for the treatment of wounds, ulcers, and inflammatory conditions⁵. Phytochemical investigations reveal that the plant contains bioactive compounds such as flavonoids, alkaloids, phenolics, and acetogenins, which contribute to its anti-inflammatory, antioxidant, and antimicrobial properties⁶. Similarly, *Ocimum sanctum* (Tulsi), a revered medicinal plant in Ayurveda, is well known for its broad pharmacological activities, including anti-inflammatory, immunomodulatory, and

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adaptogenic effects⁷. Its leaves are rich in essential oils, flavonoids, and phenolic compounds that modulate inflammatory mediators and oxidative stress pathways⁸. Polyherbal formulations, which combine extracts from multiple plants, are believed to offer synergistic therapeutic benefits by targeting multiple mechanisms simultaneously⁹. The combination of *Annonasquamosa* and *Ocimum sanctum* may therefore enhance anti-inflammatory efficacy through complementary phytoconstituents¹⁰.

The present study aims to evaluate the anti-inflammatory potential of orally administered polyherbal leaf extracts of *Annonasquamosa* and *Ocimum sanctum* in experimental rat models. By assessing their effects on induced inflammation, this research seeks to provide scientific validation for traditional claims and explore the potential of these plants as natural alternatives to synthetic anti-inflammatory agents.

MATERIAL AND METHODS

Collection of plant material: The leaves of plant *Annonasquamosa* and *Ocimum sanctum* were collected locally from Jodhpur, Rajasthan, India.

Authentication of plant material: The plant *A.squamosa* and *O.sanctum* was authenticated via order No.: भा.व.स./शु.आ.क्ष.के./A.12012/Tech.2024-25(PI.Id)/520 dated 01/11/2024 of Botanical Survey of India, (Ministry of Environment, Forest and Climate Change) Arid Zone Regional Centre, Jodhpur, Rajasthan, India.

Acute oral toxicity study: Acute oral toxicity study will be conducted according to OECD guideline 423 ANNEX 2c.

The acute toxicity of the plant extract was assessed by estimating the median lethal dose (LD₅₀) following a modified protocol based on Lorke's¹¹ method. The experiment was conducted in two phases using a total of eighteen mice. The animals were divided into six groups, each consisting of three mice. Different doses of poly-herbal extract (200 and 2000 mg/kg body weight) were administered orally. After dosing, the mice were closely monitored for 24 hours to record any mortality, neurological alterations, or behavioral signs indicative of toxicity¹². The LD₅₀ value of the extract was then calculated using the following formula:

$$LD_{50} =$$

$$\sqrt{\frac{\text{Highest dose that produced no mortality} \times \text{lowest dose that produced mortality}}{\text{Anti-inflammatory activity \%}}} = \frac{(1-D/C) \times 100}{100}$$

Study design: Three polyherbal formulations were prepared by the extract of *Annonasquamosa* and *Ocimum sanctum*.

- Test sample A: (25:75) extract of *Annonasquamosa* and *Ocimum sanctum* (200mg/Kg Body weight of extracts)
- Test sample B: (50:50) extract of *Annonasquamosa* and *Ocimum*

sanctum (200mg/Kg Body weight of extracts)

- Test sample C: (75:25) extract of *Annonasquamosa* and *Ocimum sanctum* (200mg/Kg Body weight of extracts)

Animals care and Handling: The animal experimental protocol was approved by the "Institutional Animals Ethical Committee (IAEC) of "BilwalMedchem and Research Laboratory Pvt. Ltd. SKS Reengus Industrial Area, Rajasthan (CCSEA registration No: 2304/PO/Rc/2024/CCSEA)" via Approval Ref Number BMRL/IAEC/2025/03-07 dated 26/03/2025. Female Wistar albino rats were provided BilwalMedchem and Research Laboratory Pvt. Ltd., Rajasthan, India. The animals were housed in standard conditions of temperature (25±2°C) and 12:12 h light-dark cycle. The rats were fed with commercial diet and water *ad Libitum*.

Determination of in-vivo anti-inflammatory activities using carrageenan induced paw edema volume: Male albino rats weighing between 200–250 g were housed under controlled room temperature conditions with regulated lighting. Prior to experimentation, the animals were fasted for at least 12 hours while maintaining free access to water. A 1% carrageenan solution was prepared using 0.9 % normal saline and diclofenac sodium was dispersed in normal saline to serve as the standard drug. A total of 36 rats were randomly divided into six groups, each consisting of six animals. Before injection, the plantar surface of the right hind paw was cleaned with 70% alcohol. The normal control group (Group I) received an injection of normal saline into the right hind paw, while Groups II to VI were administered 0.1 ml of 1% carrageenan solution sub-plantar to induce edema. One hour prior to carrageenan injection, Group III was treated with diclofenac sodium dispersion, Group IV received a dispersion containing 25% ASME and 75% OSWE, Group V was treated with a 50% ASME and 50% OSWE mixture, and Group VI was administered a dispersion of 75% ASME and 25% OSWE. Paw volume (ml) was measured by water displacement with a plethysmometer, 0.5 h, 1 h, 2 h, and 3 h after treatment with carrageenan. The percentage was calculated^{13, 14} by the following equation:

$$\text{Anti-inflammatory activity \%} = \frac{(1-D/C) \times 100}{100}$$

Where, D represents the difference in paw volume before and after drug administration to the rats and C represents the difference of volume in the control groups. Animals were anesthetized. Tissue punch of 6 mm diameter was collected for edema measurement and histology.

Determination of in-vivo anti-inflammatory activities using egg albumin induced

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inflammation in Wistar rats: Wistar rats were allocated into six groups of six animals each, based on their initial paw volume measurements. Treatments were administered orally in a single dose, one hour prior to inflammation induction, using aqueous leaf extracts of the selected plants. Group I served as the disease control and received 0.5% carboxymethyl cellulose (CMC). For preparation, the required amount of each test extract was accurately weighed using an analytical balance, placed in a mortar, and triturated with the appropriate volume of CMC to achieve a final concentration of 20 mg/ml. Diclofenac sodium at a dose of 20 mg/kg in 0.5% CMC was used as the standard reference drug for comparison. All treatments, including vehicles, test extracts, and the standard drug, were administered orally in a single dose one hour prior to the injection of egg albumin. Inflammation was induced by injecting 0.1 ml of freshly prepared, undiluted egg albumin into the sub-plantar region of the left hind paw. Paw volume was recorded using a plethysmometer at baseline (0 hour) and subsequently at one-hour intervals for a total duration of four hours following the injection. The increase in paw volume, calculated as the difference between the final and initial measurements, was taken as the edema volume, serving as an index of inflammation. The percent inhibition of oedema volume was calculated using the following^{15,16}:

$$\text{Paw oedema} = (V_t - V_0)$$

V_0 = Paw oedema at time zero
 V_t = Paw oedema at time t (0.5, 1, 2, 3, 24h)

$$\text{Percentage inhibition of oedema} = \frac{(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated groups}}}{(V_t - V_0)_{\text{control}}} \times 100$$

Cytokines Assay: IL-10 (anti-inflammatory cytokines) and IL-6, TNF- α (proinflammatory cytokines) in picogram per millilitre (pg/ml) were estimated with the help of ELISA Reader (Lisa Plus, Germany). Serum samples were used. IL-6 and TNF- α (Ray Bio) and IL-10 (DNA bio) ELISA kits were used. Assays were performed according to the manufacturer's recommendations.

Histopathological Studies: A 10% buffered formaldehyde solution was employed to preserve paw and liver biopsy samples. Following dehydration, the tissues were embedded in paraffin, and thin sections of 5 μ m were prepared. These sections were stained using hematoxylin and eosin and mounted on glass slides. Microscopic examination was carried out under light microscopy at 100x and 400x magnifications using an Olympus microscope equipped with a Nikon DS-Fi2 camera, enabling assessment of edema severity and inflammatory cell infiltration within the keratin layer. For ear biopsies, each sample was analyzed at ten representative sites and weight of the inflamed tissue punches was measured in micrograms, serving as an index of edema.

RESULTS AND DISCUSSION

Acute toxicity study

The first phase of the acute oral toxicity study, the groups of mice that received polyherbal extract formulations at doses of 200 mg/kg and 2000 mg/kg showed no signs of mortality. Throughout the observation period, the animals remained normal in behavior and exhibited no neurological or toxic manifestations. These findings indicate that the extract was non-toxic at the tested doses, as summarized in the table below:

Table No.1: Acute toxicity profile of received polyherbal extract formulations

Group	Poly herbal formulation	Dose 200mg/Kg	Mortality rate	Behavioral signs
Group-I	Test sample A: (25:75)	200	0/3	0/3
Group-II	Test sample A: (25:75)	2000	0/3	0/3
Group-III	Test sample B: (50:50)	200	0/3	0/3
Group-IV	Test sample B: (50:50)	2000	0/3	0/3
Group-V	Test sample C: (75:25)	200	0/3	0/3
Group-VI	Test sample C: (75:25)	2000	0/3	0/3

Anti-inflammation activity using carrageenan induced paw edema volume

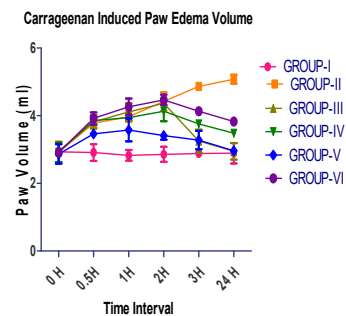


Figure 1: Anti-inflammation activity using Paw edema volume

Data are expressed as mean \pm SEM (n=6), Values along groups were statistically significant at P<

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0.05. The ^aP< 0.05 Vs Control (Group-I), The ^bP< 0.05 Vs Carrageenan (Group-II), Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

Diclofenac sodium (Group III) demonstrated the strongest anti-inflammatory effect, validating the experimental design. The 50:50 ASME:OSWE combination (Group V) showed significant efficacy, comparable to diclofenac at later stages, suggesting a promising natural alternative. The 25:75 ratio (Group IV) exhibited moderate activity, while the 75:25 ratio (Group VI) was largely ineffective, highlighting the importance of extract proportion in determining therapeutic potential. The study demonstrates that carrageenan induces significant paw edema, which can be effectively suppressed by diclofenac sodium and, notably, by a balanced 50:50 combination of ASME and OSWE. These findings suggest that the synergistic interaction between the two extracts plays a crucial role in modulating inflammation, and further phytochemical investigations could help identify the active constituents responsible for this effect.

Weight difference of inflamed tissue punch: Carrageenan markedly increased tissue weight, confirming inflammation. Diclofenac sodium served as a strong positive control, reducing edema effectively. Among the extract combinations, the 50:50 ASME:OSWE ratio (Group V) demonstrated the greatest efficacy, approaching diclofenac's effect and showing superiority over other ratios. The 25:75 and 75:25 ratios showed moderate activity, suggesting that extract proportion critically influences anti-inflammatory outcomes. The findings indicate that carrageenan-induced inflammation can be significantly attenuated by diclofenac sodium and by a balanced 50:50 combination of ASME and OSWE. The superior performance of the 50:50 ratio suggests a synergistic effect between the two extracts, making it a promising candidate for further investigation as a natural anti-inflammatory agent. Future studies should focus on isolating active phytoconstituents and elucidating the mechanisms underlying this synergism.

Weight of inflamed tissue punch in carrageenan induced rats

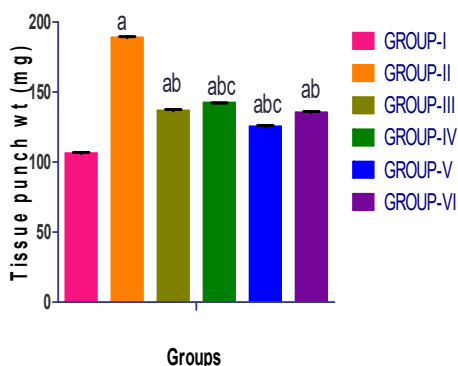


Figure 2: Weight of inflamed tissue punch in carrageenan induced rats

Data are expressed as mean ± SEM (n=6 animals),***Values along groups werestatistically significant at P< 0.05. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

% Weight difference of inflamed tissue punch in carrageenan induced rats

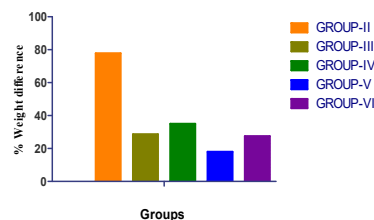


Figure 3: % Weight difference of inflamed tissue punch in carrageenan induced rats

Effects of Polyherbal formulation on cytokines expression: Carrageenan strongly upregulated pro-inflammatory cytokines (IL-6, TNF-α) and suppressed IL-10. Diclofenac sodium effectively reversed these changes, confirming its anti-inflammatory potency. Among the polyherbal formulations, the 50:50 ASME:OSWE ratio (Group V) demonstrated the greatest efficacy, closely resembling diclofenac's effect by reducing pro-inflammatory cytokines and restoring IL-10. The 25:75 and 75:25 ratios showed moderate activity, highlighting the importance of extract proportion in determining therapeutic potential. The study demonstrates that carrageenan-induced inflammation is characterized by elevated IL-6 and TNF-α with suppressed IL-10. Diclofenac sodium effectively modulates these cytokines, and notably, the 50:50 ASME:OSWE polyherbal formulation exhibits comparable efficacy. This suggests a synergistic interaction between the two extracts, making the balanced formulation a promising candidate for natural anti-inflammatory therapy. Further mechanistic studies are warranted to identify active phytoconstituents responsible for cytokine regulation.

Effects on IL-6 in carrageenan induced rats

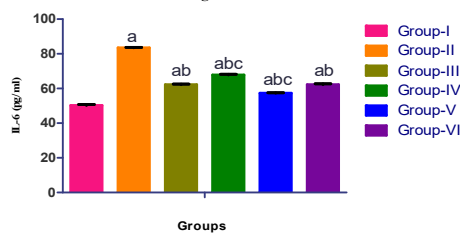


Figure 4: Effects on IL-6 in carrageenan induced rats

Data are expressed as mean ± SEM (n=6 animals),***Values along groups werestatistically significant at P< 0.0001. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

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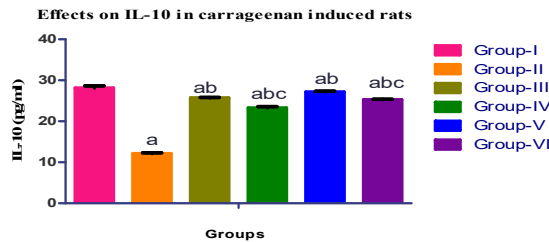


Figure 5: Effects on IL-10 in carrageenan induced rats

Data are expressed as mean ± SEM (n=6 animals), ***Values along groups werestatistically significant at P< 0.0001. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

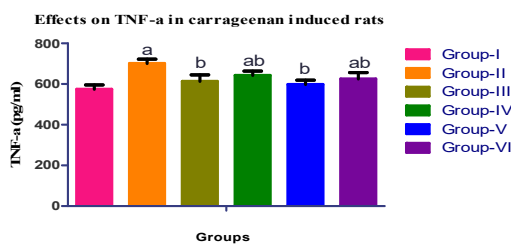


Figure 6: Effects on TNF-α in carrageenan induced rats

Data are expressed as mean ± SEM (n=6 animals), ***Values along groups werestatistically significant at P< 0.0001. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

Histopathological studies of carrageenan induced rats paw skin: The histopathological analysis of paw tissue of carrageenan treated group shows massive inflammation and edema formation compared to control group animals. Inflammatory cell infiltration, proliferated epithelium, proliferated collagen, epidermal edema was markedly suppressed by polyherbal formulations (Group-IV to Group-VI) and Diclofenac sodium administrated group as compared to carrageenan-treated group.

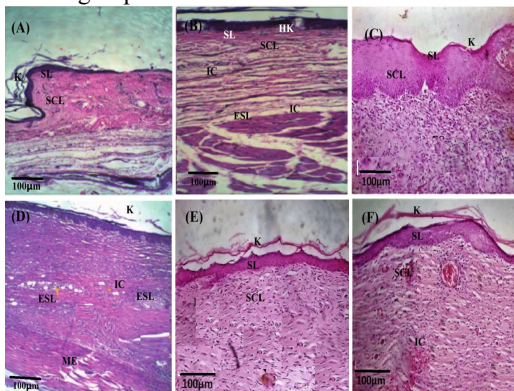


Figure 7: Histopathology carrageenan induced rat paw skin tissues in

(A) Control (B) Carrageenan (C) Diclofenac sodium (D) (25%ASME:75%OSWE) (E) (50%ASME:50%OSWE) (F) (75%ASME:25%OSWE). Where, keratin (K), Subepidermal layer (SL) subcutaneous layer (SCL), influx of inflammatory cell (IC), hyper keratinization (HK), edema in sub epidermal region (ESL), mild edema (ME), epithelial hyper plasia (EHP)

Anti-inflammation activity using egg albumin induced paw edema volume

Egg albumin significantly increased paw volume, confirming inflammation. Diclofenac sodium effectively suppressed edema, validating its role as a standard anti-inflammatory agent. Among the polyherbal formulations, the 50:50 ASME:OSWE ratio (Group V) demonstrated the greatest efficacy, closely resembling diclofenac's effect. The 25:75 and 75:25 ratios showed moderate activity, suggesting that extract proportion critically influences therapeutic potential. The study demonstrates that egg albumin-induced paw edema can be effectively attenuated by diclofenac sodium and, notably, by a balanced 50:50 combination of ASME and OSWE. The superior performance of the 50:50 ratio suggests a synergistic interaction between the two extracts, making it a promising candidate for natural anti-inflammatory therapy. Further investigations into the phytoconstituents and mechanisms of action are warranted to substantiate these findings.

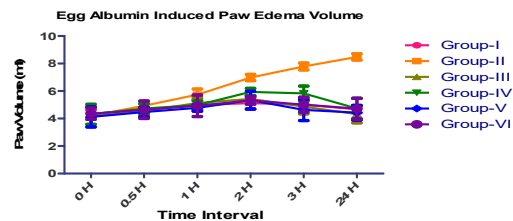


Figure 8: Effect of extracts on egg albumin-induced rat paw oedema

Data are expressed as mean ± SEM (n=6), Values along groups werestatistically significant at P< 0.05. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

Weights of inflamed tissue punch of egg albumin-induced rats: Egg albumin markedly increased tissue weight, confirming inflammation. Diclofenac sodium served as a strong positive control, reducing edema effectively. Among the extract combinations, the 50:50 ASME:OSWE ratio (Group V) demonstrated the greatest efficacy, approaching diclofenac's effect and showing superiority over other ratios. The 25:75 and 75:25 ratios showed moderate activity, suggesting that extract proportion critically influences anti-inflammatory outcomes. The findings indicate that egg albumin-induced inflammation can be significantly attenuated by

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diclofenac sodium and by a balanced 50:50 combination of ASME and OSWE. The superior performance of the 50:50 ratio suggests a synergistic effect between the two extracts, making it a promising candidate for natural anti-inflammatory therapy. Further studies should focus on isolating active phytoconstituents and elucidating the mechanisms underlying this synergism.

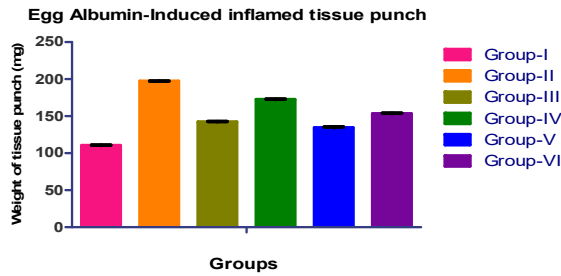


Figure 9: Effect of Extracts on Egg Albumin-Induced inflamed tissue punch

Data are expressed as mean ± SEM (n=6 animals),***Values along groups werestatistically significant at P< 0.0001. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

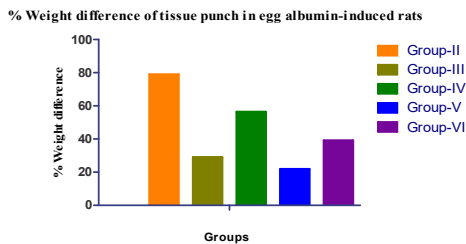


Figure 10: % Weight difference of tissue punch in egg albumin-induced rats

Cytokines expression in egg albumin-induced rats: Egg albumin strongly upregulated pro-inflammatory cytokines (IL-6, TNF- α) and suppressed IL-10. Diclofenac sodium effectively reversed these changes, confirming its anti-inflammatory potency. Among the polyherbal formulations, the 50:50 ASME:OSWE ratio (Group V) demonstrated the greatest efficacy, closely resembling diclofenac's effect by reducing pro-inflammatory cytokines and restoring IL-10. The 25:75 and 75:25 ratios showed moderate activity, highlighting the importance of extract proportion in determining therapeutic potential. The study demonstrates that egg albumin-induced inflammation is characterized by elevated IL-6 and TNF- α with suppressed IL-10. Diclofenac sodium effectively modulates these cytokines, and notably, the 50:50 ASME:OSWE polyherbal formulation exhibits comparable efficacy. This suggests a synergistic interaction between the two extracts, making the balanced formulation a promising candidate for natural anti-inflammatory therapy.

Further mechanistic studies are warranted to identify the active phytoconstituents responsible for cytokine regulation.

Effects on IL-6 in egg albumin-induced rats

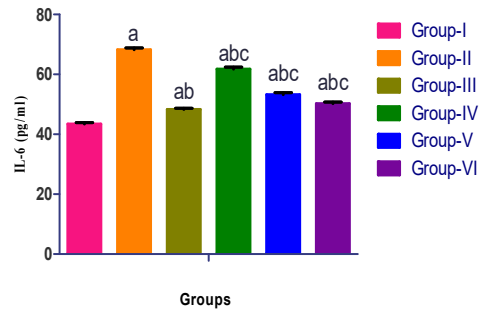


Figure 11: Effects of polyherbal formulation on IL-6 in egg albumin-induced rats

Data are expressed as mean ± SEM (n=6 animals),***Values along groups werestatistically significant at P< 0.0001. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

Effects of IL-10 in egg albumin-induced rats

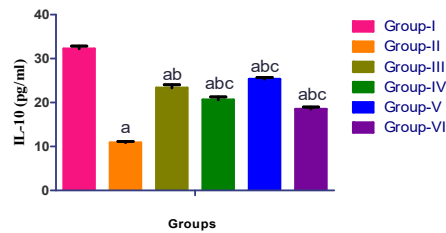


Figure 12: Effects of polyherbal formulation on IL-10 in egg albumin-induced rats

Data are expressed as mean ± SEM (n=6 animals),***Values along groups werestatistically significant at P< 0.0001. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison test

Effects of on TNF- α in egg albumin-induced rats

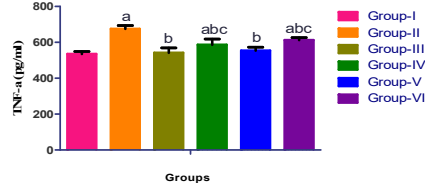


Figure 13: Effects of polyherbal formulation on TNF- α in egg albumin-induced rats

Data are expressed as mean ± SEM (n=6 animals),***Values along groups werestatistically significant at P< 0.0001. Data analyzed using One-wayANOVA followed byTukey'spost hoc comparison Test

Histopathological Studies: Histopathological evaluation confirmed that carrageenan induced severe inflammatory changes, while diclofenac significantly reduced tissue damage. Polyherbal formulations demonstrated varying degrees of protection, with the 50:50 ASME:OSWE ratio

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(Group V) showing the most favorable outcome, reducing edema and hyperplasia more effectively than other ratios. This suggests a synergistic effect of the balanced formulation in mitigating inflammation.

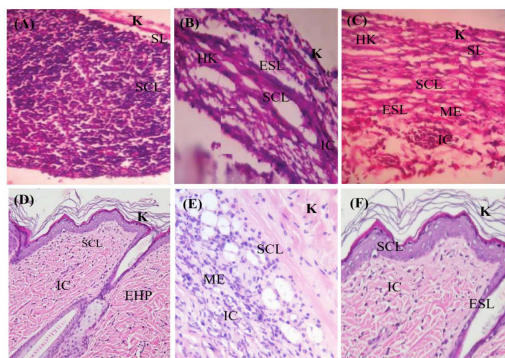


Figure 14: Histopathology of rat paw skin tissues
 (A) Control (B) Carrageenan (C) Diclofenac sodium (D) (25%ASME:75%OSWE) (E) (50%ASME:50%OSWE) (F) (75%ASME:25%OSWE). Where, keratin (K), Subepidermal layer (SL) subcutaneous layer (SCL), influx of inflammatory cell (IC), hyperkeratinization (HK), edema in sub epidermal region (ESL), mild edema (ME), epithelial hyperplasia (EHP)

CONCLUSION

The present study highlights the anti-inflammatory potential of a polyherbal formulation prepared from the leaves of *Annonasquamosa* and *Ocimum sanctum*, collected and authenticated from Jodhpur, Rajasthan, India. Experimental evaluation using carrageenan- and egg albumin-induced rat paw edema models demonstrated that diclofenac sodium served as a strong positive control, effectively reducing paw volume, tissue weight, and modulating cytokine expression. Among the polyherbal formulations, the 50:50 ratio of ASME:OSWE (Group V) consistently exhibited the greatest efficacy, significantly attenuating edema, reducing tissue punch weight, downregulating pro-inflammatory cytokines (IL-6, TNF- α), and restoring anti-inflammatory cytokine IL-10 levels. Histopathological analysis further confirmed that this balanced ratio minimized edema and epithelial hyperplasia more effectively than other combinations. In contrast, the 25:75 and 75:25 ratios showed moderate activity, underscoring the importance of extract proportion in determining therapeutic outcomes. Overall, the findings suggest that the synergistic interaction between ASME and OSWE at equal proportions plays a crucial role in modulating inflammation, making the 50:50 formulation a promising natural alternative to conventional anti-inflammatory drugs. Future research should focus on isolating the active phytoconstituents, exploring molecular mechanisms of action, and validating efficacy through advanced pharmacological and clinical

studies to establish this formulation as a potential therapeutic agent.

CONFLICTS OF INTERESTS

There are no any conflicts of interests.

REFERENCES

1. Fullerton, J. N. & Gilroy, D. W. Resolution of inflammation: a new therapeutic frontier. *Nat. Rev. Drug Discov.* **15**, 551–567 (2016).
2. Pravin V. Gomase, Priti S. Shire, SayyedNazim and Amol B. Choudhari, Development and evaluation of polyherbal formulation for anti-inflammatory activity, *J. Nat. Prod. Plant Resour.*, 2011, 1 (1): 85-90.
3. Bender, E.C., Tareq, H.S. & Suggs, L.J. Inflammation: a matter of immune cell life and death. *npj Biomed. Innov.* **2**, 7 (2025).
4. Bhattacharya A., & Chakraverty R. (2016). The pharmacological properties of *Annonasquamosa* Linn: A review. *International Journal of Pharmacy and Engineering*, 4(2), 692–699.
5. Bonneau N., Baloul L., Bajin Ba Ndob I., Sénéjoux F., & Champy P. (2017). The fruit of *Annonasquamosa* L. as a source of environmental neurotoxins: From quantification of squamocin to annotation of annonaceous acetogenins by LC-MS/MS analysis. *Food Chemistry*, 226, 32–40.
6. Chavan M. J., Wakte P. S., & Shinde D. B. (2010). Analgesic and anti-inflammatory activity of caryophyllene oxide from *Annonasquamosa* L. bark. *Phytomedicine*, 17(2), 149–151.
7. Roshan Kumar, Purabi Shah, (2022) A Systemic Review of *Ocimum sanctum* (Tulsi): Morphological Characteristics, Phytoconstituents Applications, and International Research in Applied Therapeutic Journal Sciences for and Biotechnology, 9(2), 221-226.
8. Mohammad Altamash, KaifKazi, ShaikhMukrram, AkashKundhare, Abdullah Danish, Accelerating Drug Discovery and Development Through Artificial Intelligence: Challenges and Opportunities, *Int. J. of Pharm. Sci.*, 2025, Vol 3, Issue 6, 3172-3178.
9. Kaelen. Synergistic effects of polyherbal formulations in phytomedicine: A review of traditional and contemporary evidence. *Journal of Phytopharmacology and Phytomedicine* 2025; 2(1): 10-13.
10. Rastogi S, Pandey MM, Rawat AK. Traditional herbs: A remedy for cardiovascular disease. *Phytomedicine*. 2016;23(11):1082-1089.

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11. Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol.* 1983;54(4):275–87.
12. Winter CA, Risley EA, Nuss GW. Carrageenin-induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med.* 1962;111(3):544–7.
13. R.B. Oliveira, D.A. Chagas-Paula, A. Secatto, T.H. Gasparoto, L.H. Faccioli, A.P. Campanelli, F.B. da Costa, Topical anti-inflammatory activity of yacon leaf extracts, *Braz. J. Pharmacogn.* 2013;23(3):497-505.
14. Yogesh HS, Kichadi SC, Muchandi IS, Gopalakrishna B. Evaluation of Anti-Inflammatory activity of *Cynodon dactylon* Pers. *India J Nut Prod Resour.* 2013;4(2):151–4.
15. Kim JH, Song H, Kim HW, Lee WY. Effects of egg white consumption on immune modulation in a mouse model of trimellitic anhydride-induced allergy. *Korean J Food Sci Anim Resour.* 2015;35:398-405.
16. Patil KR, Mahajan UB, Unger BS, Goyal SN, Belemkar S, Surana SJ, et al. Animal models of inflammation for screening of anti-inflammatory drugs: Implications for the discovery and development of phytopharmaceuticals. *Int J Mol Sci.* 2019;20:1-38.