Exploring the Synergistic Anti-Arthritic Effects of Boswellia serrata and Inula racemosa in CFA-Induced Inflammatory Arthritis: An In-vivo study

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Abstract

Background:

Herbal remedies such as Boswellia serrata and Inula racemosa have been traditionally employed in the management of inflammatory disorders, notably arthritis. However, the combined efficacy of these botanicals and their potential interactions remain insufficiently elucidated.

Objectives:

To investigate the phytochemical profiles and anti-arthritic potential of Boswellia serrata and Inula racemosa, individually and in combination, employing LC-MS, FTIR spectroscopy, and the CFA-induced arthritis model in Wistar rats.

Methods:

Phytochemical characterization was performed using Liquid Chromatography-Mass Spectrometry (LC-MS) and Fourier Transform Infrared (FTIR) spectroscopy. The in vivo anti-arthritic activity was assessed in Wistar rats via the Complete Freund's Adjuvant (CFA)-induced arthritis model. Hematological and biochemical parameters were evaluated, and radiographic imaging was utilized to assess joint morphology.

Results:

LC-MS analysis identified key constituents including boswellic acid derivatives (KBA, AKBA, α-BA, β-BA, AB-BA) and sesquiterpene lactones (alantolactone, iso-alantolactone). FTIR spectra confirmed the presence of characteristic functional groups consistent with these phytoconstituents. Boswellia serrata (50 mg/kg) exhibited pronounced anti-inflammatory effects, whereas Inula racemosa did not demonstrate comparable activity. Notably, their combination resulted in an antagonistic interaction, diminishing the efficacy observed with Boswellia serrata alone. Hematological analysis revealed reductions in mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in the combination group, suggestive of microcytic anaemia. Biochemical assays indicated reduced levels of alkaline phosphatase (ALP), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT), implying hepatoprotective and anti-inflammatory benefits. Radiographic examination confirmed preservation of joint architecture without evidence of cartilage erosion or bony deformities in treated cohorts.

Conclusion:

Boswellia serrata demonstrated superior anti-arthritic efficacy as a monotherapy. Its combination with Inula racemosa appears to exert a counterproductive interaction, potentially of pharmacokinetic or pharmacodynamic nature. Further molecular and clinical investigations are warranted to elucidate these interactions and optimise their therapeutic potential in arthritis management.

Highlights

- LC-MS analysis confirmed the presence of key boswellic acid derivatives (KBA, AKBA, α-BA, β-BA, AB-BA) in Boswellia serrata, and sesquiterpene lactones (alantolactone and iso-alantolactone) in Inula racemosa, based on retention times and mass-to-charge (m/z) ratios.
- FTIR spectroscopy validated the structural heterogeneity of phytoconstituents through the identification of hydroxyl, carbonyl, aliphatic, aromatic, and amine functional groups.
- Boswellia serrata (50 mg/kg) significantly attenuated paw oedema and ankle diameter in CFA-induced arthritic rats, confirming its robust anti-inflammatory potential.

- Inula racemosa, when administered alone, failed to demonstrate any significant anti-inflammatory activity.
- Co-administration of Boswellia serrata and Inula racemosa resulted in a marked reduction in the therapeutic efficacy of Boswellia serrata, suggesting a possible pharmacokinetic or pharmacodynamic antagonism.
- Combination therapy led to decreased serum levels of ALP, SGOT, and SGPT, indicating hepatoprotective and anti-inflammatory effects.
- Radiographic analysis showed preservation of joint architecture across treated groups, with no observable cartilage loss or bony deformities.

Introduction

Arthritis represents a chronic inflammatory disorder of the joints, primarily characterised by synovial membrane inflammation, pain, and restricted mobility (Satapathy et al., 2025). Among its various clinical manifestations, rheumatoid arthritis (RA) stands out as a common autoimmune condition, marked by symmetrical joint involvement, progressive cartilage degradation, and eventual deformity (Mishra, 2025; Patel et al., 2025). Although conventional pharmacological interventions such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs) offer symptomatic relief, their long-term administration is often constrained by adverse effects and limited tolerability. This highlights the pressing need for safer, plant-derived alternatives (Ghosh et al., 2024; Meher et al., 2012). The present study investigates the anti-arthritic efficacy and interaction potential of Boswellia serrata (family: *Burseraceae*) and Inula racemosa (family: *Asteraceae*), both individually and in combination. Boswellia serrata resin is rich in boswellic acids such as 11-keto-β-boswellic acid (KBA) and 3-O-acetyl-11-keto-β-boswellic acid (AKBA) which are known to inhibit 5-lipoxygenase activity and modulate pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), thereby contributing to its anti-inflammatory and chondroprotective properties (Solanki et al., 2024). Conversely, Inula racemosa roots contain sesquiterpene lactones such as alantolactone and iso-alantolactone which have been reported to suppress nuclear factor-kappa B (NF-κB) activation and mitigate inflammatory responses (Santoshrao et al., 2025). To confirm the phytochemical composition of the plant extracts, Fourier Transform Infrared (FTIR) spectroscopy and Liquid Chromatography-Mass Spectrometry (LC-MS) were employed, revealing the presence of key bioactive markers and functional groups. Subsequent *in vivo* evaluation using the Complete Freund's Adjuvant (CFA)-induced arthritis model in Wistar rats demonstrated a significant anti-inflammatory effect of *Boswellia serrata* at 50 mg/kg. In contrast, *Inula racemosa* failed to produce comparable therapeutic outcomes. Intriguingly, the co-administration of both extracts attenuated the beneficial effects of *Boswellia serrata*, indicating a potential antagonistic interaction of either pharmacokinetic or pharmacodynamic origin. Haematological and biochemical analyses further revealed alterations in mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), along with reductions in alkaline phosphatase (ALP), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT) levels. These findings suggest both therapeutic relevance and potential safety concerns in the context of combination therapy. In summary, this investigation validates the individual anti-arthritic efficacy of *Boswellia serrata* and underscores the necessity for detailed mechanistic studies to elucidate the nature of its antagonistic interaction with Inula racemosa, thereby guiding the rational design of optimised polyherbal formulations for the effective management of arthritis.

Materials and Methods

Chemicals and reagents

Complete Freund's Adjuvant (CFA) was obtained from Sigma-Aldrich, USA; picric acid was sourced from Suvidhinath Laboratories Pvt. Ltd.; ethanol from Loba Chemie, USA; and halothane from Piramal Enterprises Limited. Ibuprofen was procured from Abbott India Limited. All chemicals and reagents employed in the study were of analytical grade and used without further purification.

Plant materials and drugs

Standardized herbal extracts of *Boswellia serrata* and *Inula racemosa* were kindly provided as gift samples by Sunpure Extracts Pvt. Ltd.

***In- vitro* characterization:**

In the present study, a preliminary investigation was undertaken to quantify the total flavonoid, alkaloid, and terpenoid contents of the selected herbal extracts. Furthermore, potential phytochemicals were identified and characterized using Fourier Transform Infrared (FT-IR) spectroscopy and Liquid Chromatography-Mass Spectrometry (LC-MS) analyses.

***In- vivo* anti- inflammatory and anti- arthritic activities:**

Experimental animals

Male albino Wistar rats, weighing between 180-200 g, were procured from the Institutional Animal House Facility, Columbia Institute of Pharmacy, Raipur (Chhattisgarh). The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. CIP/IAEC/2025/251; Registration No. 1321/PO/ReBi/10/CPCSEA). Prior to the commencement of the experiments, the animals were acclimatized for a period of two weeks under standard laboratory conditions. The rats were housed in polypropylene cages with husk bedding and maintained under a controlled environment with a 12-hour light/dark cycle and regulated relative humidity, in accordance with CPCSEA guidelines. A standard pelleted diet and Aqua Guard-purified water were provided *ad libitum* throughout the experimental duration. Animals were monitored regularly for any signs of behavioral abnormalities or distress.

Experimental design

Adult male Wistar rats, weighing between 180–200 g, were randomly assigned to six groups (n = 6 per group) based on body weight:

Group I – Normal control group

Group II – Arthritis-induced group (arthritic control)

Group III – *Boswellia serrata*-treated group (50 mg/kg, orally)

Group IV – *Inula racemosa*-treated group (100 mg/kg, orally)

Group V – Combination-treated group receiving *Boswellia serrata* (25 mg/kg) and *Inula racemosa* (50 mg/kg), orally

Group VI – Ibuprofen-treated group (72 mg/kg, orally)

Induction of Arthritis and Treatment Schedule in Experimental Rats:

Arthritis was induced in experimental rats in accordance with the method described by H. Gerhard Vogel (2008; Vogel, 2002). Briefly, 0.1 mL of Complete Freund’s Adjuvant (CFA), containing heat-killed Mycobacterium tuberculosis, was injected into the left hind paw of all animals, with the exception of those in Group I (normal control). One hour prior to CFA administration, animals in Groups III, IV, V, and VI received oral doses of *Boswellia serrata*, *Inula racemosa*, the combination of both herbal extracts, and ibuprofen (std.drug), respectively. No treatment was administered during the induction phase (days 0–7). From day 8 onwards, treatment resumed and was continued until day 28. Animals in the arthritic control group (Group II) received CFA alone (0.1 mL) without any therapeutic intervention. A 1 mL glass syringe fitted with locking hubs and a 26G needle was used for CFA injection. Due to the viscous nature of the adjuvant, administration was carried out under halothane anaesthesia to minimize animal distress and facilitate accurate injection.

Paw swelling was assessed periodically up to day 28 using mercury plethysmometry. For this, the inflamed paw was immersed in mercury up to the lateral malleolus, which had been marked with ink to ensure consistency. Ankle joint diameter was measured using a vernier caliper, as described by Kaushik et al. (2021). The change in paw volume was calculated using the following formula:

Increased volume of edema = Final paw volume − Initial paw volume

Assessment of paw volume and ankle joint diameter in experimental animals:

The progression of arthritis was monitored by measuring the paw volume and ankle joint diameter at regular intervals. Specifically, the volume of the left hind paw was recorded weekly from day 0 to day 28 in all experimental groups using a Digital Plethysmometry (Orchid Scientific & Innovative India Pvt. Ltd., Maharashtra, India), as described by Singh et al. (2021). This method enabled precise quantification of inflammation-induced edema. In parallel, the diameter of the ankle joint was measured using a calibrated vernier caliper, in accordance with the procedure established by Coderre et al. (1987). This parameter served as an additional indicator of joint swelling and inflammation severity. These measurements were instrumental in evaluating the therapeutic efficacy of the test substances by allowing a comparative assessment of inflammation across different treatment groups over the 28-day experimental period.

Determination of body weight:

Body weight changes were monitored as an indicator of general health status and systemic effects of arthritis and treatment interventions. Animals from all experimental groups were individually placed in stainless steel restrainers to ensure minimal movement during weighing. The measurements were taken on days 0, 7, 14, 21, and 28 from the day of arthritis induction. The weighing was conducted using a digital weighing balance, ensuring precision and consistency across all time points. The actual body weight of each animal was determined by subtracting the weight of the empty restrainer from the total recorded weight (animal + restrainer), following the method described by Mahdi et al. (2018). Regular assessment of body weight provided valuable insights into the disease progression and the potential impact of the administered treatments on overall physiological well-being.

Blood collection, hematological and biochemical estimation:

At the conclusion of the experimental period, i.e., on the 28th day, blood samples were collected from all animals for hematological and biochemical analyses. Collection was performed via the retro-orbital plexus under light anaesthesia, ensuring minimal discomfort to the animals, as per established protocols. The collected blood was immediately subjected to hematological evaluation. The parameters assessed included hemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), platelet count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), in accordance with the methodology described by Banji et al. (2011). These indices provided insights into the systemic inflammatory status and the potential hematological alterations induced by both disease progression and therapeutic intervention. Following hematological assessment, the remaining blood samples were centrifuged at 5000 revolutions per minute (RPM) for 10 minutes at 4 °C to obtain plasma. The separated plasma was then used for the estimation of key biochemical markers, including alkaline phosphatase (ALP), serum glutamic-pyruvic transaminase (SGPT), and serum glutamic-oxaloacetic transaminase (SGOT), as per the method outlined by Nazar et al. (2024). These enzymes served as indicators of hepatic function and systemic inflammation, providing a comprehensive biochemical profile of the treatment effects.

Radiological analysis (X- ray) of arthritic paw of experimental rats

At the end of the dosing schedule, radiological analysis was conducted to evaluate the protective effects of *Boswellia serrata* and *Inula racemosa* root extracts on joint architecture. For this purpose, one animal from each experimental group was randomly selected and subjected to X-ray examination of the affected hind paw.

Prior to radiographic imaging, animals were anaesthetised using halothanes to ensure complete immobilization and minimize discomfort during the procedure. The anaesthetized animal's hind paw was carefully positioned on a smooth, flat surface to obtain a clear and standardized radiographic view. X-ray imaging was performed using a portable digital X-ray apparatus, enabling high-resolution visualization of bone structures and joint spaces. This allowed for the assessment of pathological changes such as bone erosion, joint space narrowing, and soft tissue swelling. The radiographs were then evaluated to determine the extent of joint protection conferred by the individual and combination treatments, as described by Nazar et al. (2024).

Statistical analysis

The data for all evaluated parameters were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) in GraphPad Prism software version 10.5.0 (774), followed by Dunnett's post hoc test for multiple comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered to indicate statistical significance.

Results

In- vitro characterization:

Liquid Chromatography-Mass Spectrometry (LC-MS) enables precise identification and characterization of complex phytochemical mixtures present within herbal extracts. The LC-MS profiles of both bioactive extracts are presented in Figures 1 to 7. In *Boswellia serrata*, the most abundant and pharmacologically significant compound identified was acetyl-11-keto-β-boswellic acid (AKBA). Additional major constituents included 11-keto-β-boswellic acid (KBA), β-boswellic acid, acetyl-β-boswellic acid, and α-boswellic acid. Among these, AKBA and KBA typically represent the most prominent peaks in the LC-MS chromatograms of Boswellia serrata extracts, reflecting their high abundance and therapeutic relevance.

Table no. 1: LC-MS Interpretation of *Boswellia serrata*

Compound Name	Molecular Formula	Retention Time (min)	Ion Type	m/z Value	Neutral Mass (Da)	Ion Description	Classification / Interpretation
11-keto-β-boswellic acid (KBA)	$C_{30}H_{48}O_4$	7.027–7.481	$[M+H]^+$	473.3668	472.36	Protonated molecule	Molecular ion confirming KBA
11-keto-β-boswellic acid (KBA)	$C_{30}H_{48}O_4$	7.027–7.481	$[M+NH_4]^+$	490.3917	472.36	Ammonium adduct	Common ion in positive ESI, supportive evidence
11-keto-β-boswellic acid (KBA)	$C_{30}H_{48}O_4$	7.027–7.481	$[M+NH_4]^+$	491.3946	472.36	Ammonium adduct (isotope/replicate)	Confirms ion stability
11-keto-β-boswellic acid (KBA)	$C_{30}H_{48}O_4$	7.027–7.481	$[M+NH_4]^+$	492.3904	472.36	Isotopic peak of ammonium adduct	Isotopic confirmation
11-keto-β-boswellic acid (KBA)	$C_{30}H_{48}O_4$	7.027–7.481	$[M+Na]^+$	495.3492	472.36	Sodium adduct	Alternate adduct, supports identification
AKBA	C32H48O5	~22.5	$[M+H]^+$	513.35	512.35	Protonated molecular ion	Triterpenoid / Boswellic acid derivative
AKBA	C32H48O5	~22.5	$[M+Na]^+$	535.33	512.35	Sodium adduct ion	Confirms sodium adduct; common in ESI-MS
AKBA (Fragment)	—	~22.6	$[M–Acetyl+H]^+$	471.33	—	Loss of acetyl group	Fragment ion confirms structural feature (acetyl group)
AKBA (Fragment)	—	~22.6	$[M–COCH3–H2O+H]^+$	453.32	—	Loss of acetyl & water	Structural fragmentation; supports presence of keto group
α-Boswellic acid (α BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+H]^+$	499.3782	498.37	Protonated molecular ion	Confirms presence of α-BA
α-Boswellic acid (α-BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+NH_4]^+$	516.4100	498.37	Ammonium adduct	Common in +ESI; supports ID
α-Boswellic acid (α-BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+NH_4]^+$	517.4122	498.37	Isotopic/adduct variant	Confirmatory isotopic signal
α-Boswellic acid (α-BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+Na]^+$	521.3601	498.37	Sodium adduct	Secondary confirmation of compound
α-Boswellic acid (α-BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+Na]^+$	522.3572	498.37	Sodium adduct (isotope/variant)	Supports ID through adduct analysis

Acetyl-β-boswellic acid (AB-BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+H]^+$	499.3782	498.37	Protonated molecular ion	Confirms presence of AB-BA
AB-BA	$C_{32}H_{50}O_4$	7.615–7.934	$[M+NH_4]^+$	516.4100	498.37	Ammonium adduct	Common adduct in positive ESI
Acetyl-β-boswellic acid (AB-BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+NH_4]^+$	517.4122	498.37	Isotopic/ammonium variant	Supports ionization confirmation
Acetyl-β-boswellic acid (AB-BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+Na]^+$	521.3601	498.37	Sodium adduct	Alternate adduct; confirms molecular structure
Acetyl-β-boswellic acid (AB-BA)	$C_{32}H_{50}O_4$	7.615–7.934	$[M+Na]^+$	522.3572	498.37	Isotopic sodium adduct	Additional confirmation through isotopic peak
β-Boswellic Acid (B-BA)	$C_{30}H_{48}O_3$	8.707–8.976	$[M+H]^+$	457.3679	456.36	Protonated molecular ion	Confirms identity of β-BA
β-Boswellic Acid (B-BA)	$C_{30}H_{48}O_3$	8.707–8.976	$[M+NH_4]^+$	474.3886	456.36	Ammonium adduct	Common adduct in positive ESI
β-Boswellic Acid (B-BA)	$C_{30}H_{48}O_3$	8.707–8.976	$[M+NH_4]^+$	475.3958	456.36	Isotopic ammonium adduct	Confirms adduct identity and isotopic distribution
β-Boswellic Acid (B-BA)	$C_{30}H_{48}O_3$	8.707–8.976	$[M+NH_4]^+$	476.4059	456.36	Likely second isotope/ammonium	Additional confirmation

In *Inula racemosa*, Alantolactone and iso-alantolactone are the compounds possess strong anti-inflammatory properties and are known to modulate inflammatory pathways such as NF-κB.

Table no. 2: LC-MS Interpretation of *Inula racemosa*

Compound Name	Molecular Formula	Retention Time	Ion Type	m/z Value	Neutral Mass	Ion Description	Classification / Interpretation
Alanto-lactone	$C_{15}H_{20}O_2$	7.222 (min)	[M+H]+	233.1575	232.1499	Protonated molecule	Sesquiterpene lactone (anti-inflammatory/anti-arthritic)
			[M+Na]+	255.1291	232.1499	Sodium adduct	
			$[M+NH_4]+$	250.1821	232.1499	Ammonium adduct	
			[M+Na]+	256.1318	232.1499	Sodium adduct (repeat ion)	
			[M+Na]+	257.1415	232.1499	Sodium adduct (isotopic peak)	
			[M+H]+	234.1613	233.1537	Isotopic protonated	
Iso-alantolactone	$C_{15}H_{20}O_2$	7.222	[M+H]+	233.1575	232.1499	Protonated molecule	Sesquiterpene lactone (bioactive, anti-arthritic)
			[M+Na]+	255.1291	232.1499	Sodium adduct	
			[M+H]+	234.1613	233.1537	Isotopic protonated ion	
			$[M+NH_4]+$	250.1821	232.1499	Ammonium adduct	
			[M+Na]+	256.1318	232.1499	Sodium adduct (repeat)	
			[M+Na]+	257.1415	232.1499	Sodium adduct (isotope)	

Similarly, FTIR helps detect key functional groups, Provides structural insights about the molecular framework of herbal bioactives. The results for FTIR of both the bioactives are depicted in fig. 8.

Table No.3: FTIR Peak Interpretation of *Boswellia serrata*

Peak No.	Wavelength (cm^{-1})	Intensity	Functional Group	Vibration Type / Bond	Interpretation
1	382.89	Medium	—	Fingerprint region	Possibly C–C skeletal or bending modes
17	1041.61	Medium	C–O	Stretching	Alcohols, esters, ethers
18	1244.14	Strong	C–O, C–N	Stretching	Phenols, ethers, amines
19	1376.27	Strong	C–H (methyl)	Bending (scissoring)	Alkanes
20	1456.32	Very Strong	C=C, CH_3	Stretching / Bending	Aromatics, alkanes
25	1704.18	Very Strong	C=O	Stretching	Ketones, aldehydes, carboxylic acids, esters
28	1975.19	Strong	C=C=C or overtone	Combination/Overtone band	Possible cyclic or complex structures
31	2141.08	Medium	C≡C or C≡N	Triple bond stretching	Alkynes, nitriles
34	2358.08	Strong	O=C=O	Stretching	CO_2, isocyanates
38	2940.61	Very Strong	C–H	Stretching	Alkanes, methyl/methylene groups
41	3251.16	Medium	O–H or N–H	Stretching (broad if H-bonded)	Alcohols, phenols, amines
44	3326.39	Medium	N–H or ≡C–H	Stretching	Terminal alkynes, primary amines
54	3742.06	Weak	O–H free	Stretching	Free hydroxyl (non H-bonded)
70	4343.88	Weak	—	Possibly overtone or artifact	Less commonly assigned region

Table No.4: FTIR Peak Interpretation for *Inula racemosa*

Peak No.	Wavenumber (cm⁻¹)	Intensity (%)	Functional Group	Vibration Type / Bond	Interpretation Example
65	2892.38	62.3	C–H (alkane)	Stretching	Aliphatic C–H stretching (methyl/methylene)
66	2942.53	62.5	C–H (alkane)	Stretching	Aliphatic symmetric/asymmetric stretch
68	3264.66	56.8	O–H / N–H	Stretching (broad)	Alcohols/Phenols or amines
72	3323.49	56.9	O–H (hydroxyl)	Stretching (broad)	Hydrogen bonded O–H group
74	3741.10	82.7	O–H (free)	Stretching	Free hydroxyl group
75	3787.39	86.2	O–H / N–H (free)	Stretching	Sharp free hydroxyl or amine group
49	1700.32	59.1	C=O	Stretching	Carbonyl stretch (likely ketones/aldehydes)
50	1713.83	59.6	C=O	Stretching	Strong carbonyl (possibly esters/carboxylic acids)
35	1510.33	60.3	C=C (aromatic)	Stretching	Aromatic ring stretch
39	1557.59	59.8	N–O / C=C	Asym. Stretch / ring mode	Nitro compounds or aromatic systems
22	1197.85	55.7	C–O	Stretching	Alcohols, ethers, esters
23	1283.68	59.7	C–N / C–O	Stretching	Amines or esters
19	935.52	58.9	=C–H	Out-of-plane bending	Aromatic or alkene hydrogen
63	2358.08	67.1	C≡N / CO_2	Asymmetric stretch	Possibly CO_2 or nitrile (if confirmed by context)

In-vivo study

Effect of different treatments on body weight

Body weight was recorded at regular intervals on days 0, 7, 14, 21, and 28. In comparison to the normal control group, rats with CFA-induced arthritis exhibited a significant reduction in body weight, reflecting systemic inflammation and disease-associated metabolic stress. Administration of the test compounds resulted in a marked and dose-dependent improvement in body weight, particularly from day-14 through to day-28, when compared with the arthritic control group. These findings suggest a potential therapeutic effect in mitigating arthritis-induced weight loss. The corresponding data are illustrated in Figure 9.

Effect of different treatments on paw volume:

A progressive linear increase in paw volume was observed in CFA-induced arthritic rats compared to the normal control group throughout the duration of the study, indicating sustained inflammatory response. Treatment with the test compounds resulted in a significant, dose-dependent reduction in paw volume from day 14 to day 28, when compared with both the arthritic control and standard drug-treated groups. This attenuation of inflammation suggests that the test compounds may inhibit the release of key pro-inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interferons, and other cytokines implicated in the pathogenesis of arthritis. The detail results are presented in Fig.10.

Effect of different treatments on ankle joint diameter

The results are depicted fig. no.16. Values expressed as Mean± S.E.M by applying one way ANOVA followed by Dunnettes t Test Using Graphpad Prism Software 10.5.0(774)

Hematological Parameters

In CFA- induced arthritic rats, significantly lower Hb level and RBC count, as well as higher the WBC count, was observed in comparison with control rats. The findings are given graphic form and it is represented in fig. no. 17.

Biochemical Parameters

The increased levels of biochemical are due to muscle damage, drug administration and risk to the liver problem. In this study, the arthritic control groups' biochemical parameters showed a slight rise in both the SGOT and SGPT levels. The results are depicted in fig. no.18.

X- Ray assessment of inflamed paw of rats

The radio graphical analysis of knee joint of different groups of animals has been carried out by X-ray study. The result of X-ray have been placed as film no./ fig no.19.

Discussion and Conclusion

The present investigation was undertaken to evaluate the anti-arthritic and anti-inflammatory potential of *Boswellia serrata* and *Inula racemosa*, both individually and in combination, in a Complete Freund's Adjuvant (CFA)-induced arthritis model using Wistar rats. The study also sought to explore potential interactions between these two bioactives when co-administered.

Phytochemical Profiling. Both herbal extracts were subjected to Liquid Chromatography-Mass Spectrometry (LC-MS) analysis to identify their principal bioactive constituents. The LC-MS profile of *Boswellia serrata* revealed the presence of key pharmacologically active boswellic acids, including 11-keto-β-boswellic acid (KBA), α-boswellic acid (α-BA), acetyl-β-boswellic acid (AB-BA), and acetyl-11-keto-β-boswellic acid (AKBA). Among these, AKBA and KBA appeared as the most prominent peaks, corroborating their dominance in the extract and their therapeutic relevance. Similarly, LC-MS analysis of *Inula racemosa* identified sesquiterpene lactones, namely alantolactone and iso-alantolactone as major constituents. These compounds are well-documented for their diverse pharmacological properties. Fourier Transform Infrared (FTIR) spectroscopy further supported the phytochemical findings by identifying various functional groups such as hydroxyl, carbonyl, aliphatic, and aromatic moieties, which are indicative of structural diversity and bioactive potential within both extracts. The *in vivo* anti-arthritic activity was assessed using a CFA-induced arthritis model in Wistar rats. The findings demonstrated that *Boswellia serrata* at a dose of 50 mg/kg exhibited significant anti-inflammatory effects, as evidenced by marked reductions in paw edema and ankle joint diameter from day 14 to day 28 post-induction. These effects may be attributed to the high concentration of pentacyclic triterpenoids, especially AKBA, known for their inhibitory action on inflammatory mediators. At the molecular level, the anti-inflammatory mechanism of *Boswellia serrata* is primarily associated with the inhibition of 5-lipoxygenase (5-LOX), an enzyme critical in leukotriene biosynthesis. This inhibition leads to reduced production of pro-inflammatory leukotrienes and subsequent neutrophil infiltration. Specifically, AKBA exhibits high binding affinity to the active site of 5-LOX, thereby suppressing its catalytic activity. Additionally, *Boswellia serrata* modulates the NF-κB signalling pathway by preventing the degradation of IκB-α, which in turn inhibits the nuclear translocation of NF-κB. This down regulates the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.

Moreover, boswellic acids have demonstrated interactions with microsomal prostaglandin E synthase-1 (mPGES-1) and cyclooxygenase-2 (COX-2), particularly via KBA and AKBA, contributing to reduced prostaglandin E2 (PGE2) synthesis. They also influence Toll-like receptor 4 (TLR4)-mediated pathways and the NLRP3 inflammasome, thereby modulating innate immune responses and promoting joint protection. In contrast, *Inula racemosa* did not exhibit significant anti-inflammatory activity when administered alone. This finding was unexpected, especially considering the well-established anti-inflammatory and pharmacological activities of its key constituents alantolactone and iso-alantolactone primarily reported in *Inula helenium*. These sesquiterpene lactones are known to exert their biological effects via alkylation of cysteine residues through their α-methylene-γ-lactone moiety. This leads to modulation of redox-sensitive signalling pathways and suppression of NF-κB activity by inhibiting IκB kinase (IKK), stabilizing IκB-α, and preventing the translocation of NF-κB p65 subunits. Alantolactone and iso-alantolactone are also known to induce intracellular reactive oxygen species (ROS), contributing to mitochondrial dysfunction and apoptosis, particularly in cancer cells. The induced ROS stress activates the JNK and p38 MAPK signalling pathways, alters the Bax/Bcl-2 ratio, disrupts mitochondrial membrane potential, and promotes cytochrome c release, culminating in caspase-3 and -9 activation and programmed cell death. Additionally, these compounds inhibit STAT3 phosphorylation, thereby reducing the expression of cell survival and proliferation-related genes such as cyclin D1, survivin, and Bcl-xL. Unexpectedly, when *Boswellia serrata* and *Inula racemosa* were co-administered, the therapeutic efficacy of *Boswellia serrata* was notably reduced. The antagonistic effect was evident in both paw edema and ankle diameter measurements, where the combination treatment failed to match the anti-inflammatory efficacy of *Boswellia serrata* alone. This suggests a possible interaction, although it remains unclear whether this is pharmacokinetic or pharmacodynamics in nature. Further evaluation is warranted to delineate the mechanistic basis of this antagonism. It is possible that, Inula *racemosa* may interfere with the absorption, metabolism, or receptor binding of *Boswellia serrata* constituents, thereby diminishing their efficacy. Hematological analysis revealed that, the combination of *Boswellia serrata* and *Inula racemosa* resulted in a significant decrease in mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), suggesting the onset of microcytic anemia. The reduction in MCV implies the production of smaller red blood cells, although the exact mechanism underlying this alteration remains unidentified. The increase in MCHC, while less common, may indicate changes in hemoglobin concentration relative to cell volume in the combination-treated group. Biochemical analysis demonstrated that, the combination therapy led to reductions in liver enzymes, alkaline phosphatase (ALP: 222.83 U/L), serum glutamic-oxaloacetic transaminase (SGOT: 59.65 U/L), and serum glutamic-pyruvic transaminase (SGPT: 35.6 U/L), when compared to the arthritic control group. These findings suggest a potential hepatoprotective effect, despite the antagonism observed in anti-inflammatory outcomes. Radiographic examination conducted at the end of the study showed preservation of joint architecture in groups treated with either *Boswellia serrata* or *Inula racemosa* alone. No evidence of cartilage erosion, joint space narrowing, or bony deformities was observed, indicating protective effects at the structural level. However, in combination-treated animals, no additional radiological benefit was evident over monotherapy.

This study confirms the potent anti-arthritic and anti-inflammatory efficacy of *Boswellia serrata* in CFA-induced arthritis, attributable to its rich content of boswellic acids and their multi-targeted mechanisms of action. In contrast, *Inula racemosa* failed to demonstrate comparable activity in this model, despite its established molecular targets in other inflammatory and oncological settings. Notably, the co-administration of *Inula racemosa* with *Boswellia serrata* resulted in an unexpected antagonistic effect, reducing the efficacy of *Boswellia serrata*. While the precise nature of this interaction remains unclear, it warrants further investigation at both pharmacokinetic and pharmacodynamic levels. Additional molecular studies are essential to elucidate the underlying mechanisms and to establish the safety, efficacy, and compatibility of these bioactives in polyherbal formulations intended for arthritis management.

Abbreviations

5-LOX: 5-Lipoxygenase

AB-BA: Acetyl-β-boswellic acid

AKBA: Acetyl-11-keto-β-boswellic acid

ANOVA: Analysis of variance

B-BA: β-Boswellic Acid

BS: Boswellia serrata

CCSEA: Committee for Control and Supervision on Experiments on Animals

CFA: Complete Freund's adjuvant

COX: Cyclooxygenase

DMARDs: Disease-modifying anti-rheumatic drugs

FTIR: Fourier Transform Infrared Spectroscopy

IAEC: Institutional Animal Ethics Committee

ICAM-1: Intercellular Adhesion Molecule 1

IL-1β: Interleukin-1 beta

IR: Inula racemosa

KBA: 11-Keto-β-boswellic acid

LC-MS: Liquid Chromatography-Mass Spectrometry

LFA-1: Lymphocyte Function-Associated Antigen-1

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

MCV: Mean Corpuscular Volume

NF-κB: Nuclear factor kappa B

NSAIDs: Non-steroidal anti-inflammatory drugs

SGOT/ AST: Aspartate amino transferase

SGPT/ ALT: Alanine Aminotransferase

STAT: Signal transducers and activators of transcription

TNF-α: Tumor Necrosis Factor alpha

α-BA: α-Boswellic acid

Declarations

Ethics approval and consent to participate:

This manuscript is a research article. Experiments in animals (experimental rats) are included in this study; ethical approval from IAEC, CIP, Raipur, C.G. has been obtained.

Consent for publication:

This manuscript does not contain any personal data. Hence, no consent is required.

Conflict of interest:

The authors declare no conflicts of interest.

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Declaration of generative AI in scientific writing:

The Authors declares that the manuscript does not contain any AI generated text.

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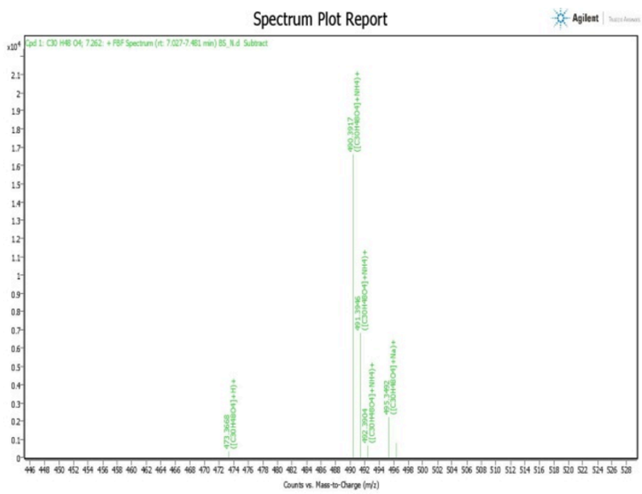
Figures



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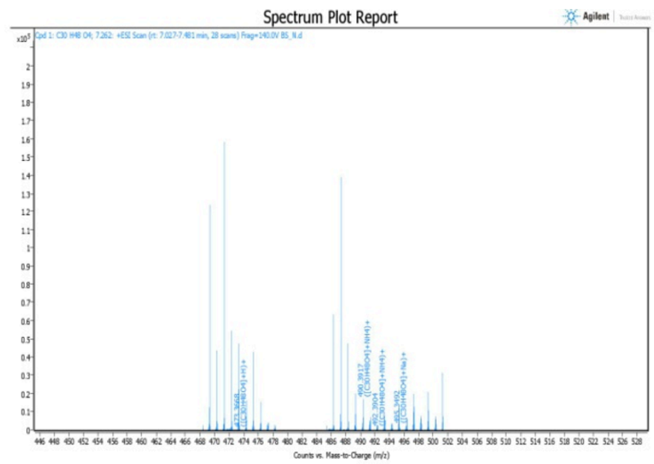
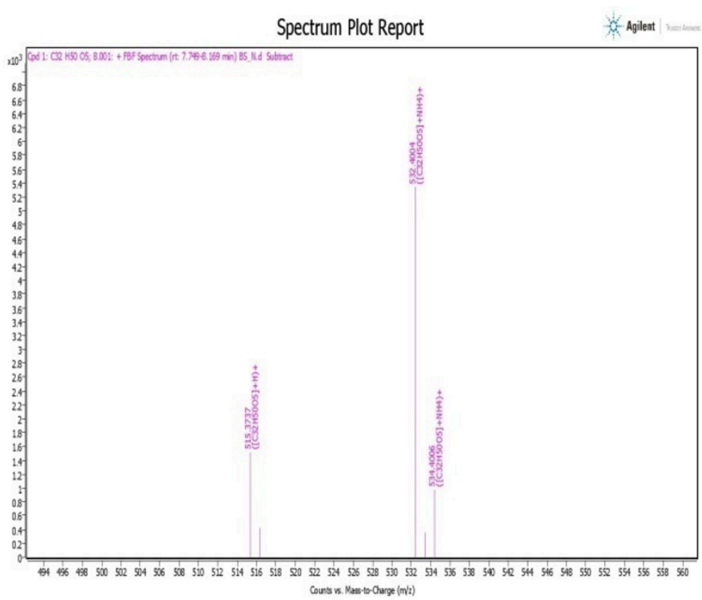


[B]

Figure 1

Graph [A] and [B] is the spectrum Plot of 11-keto-β-boswellic acid (KBA)



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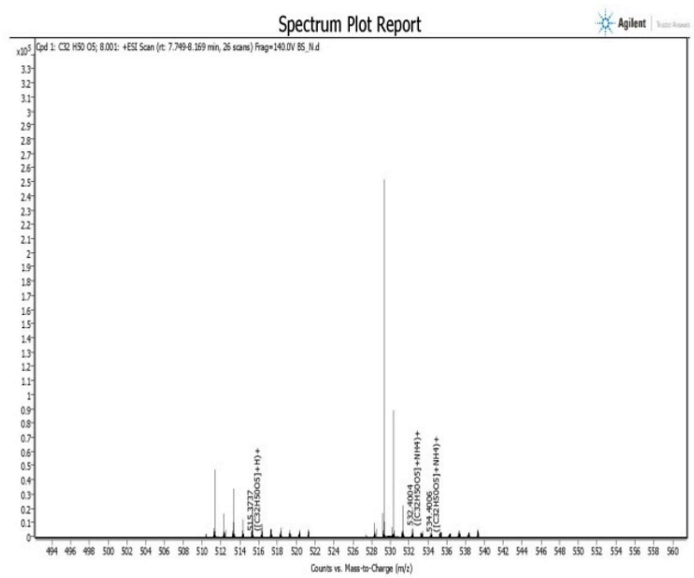
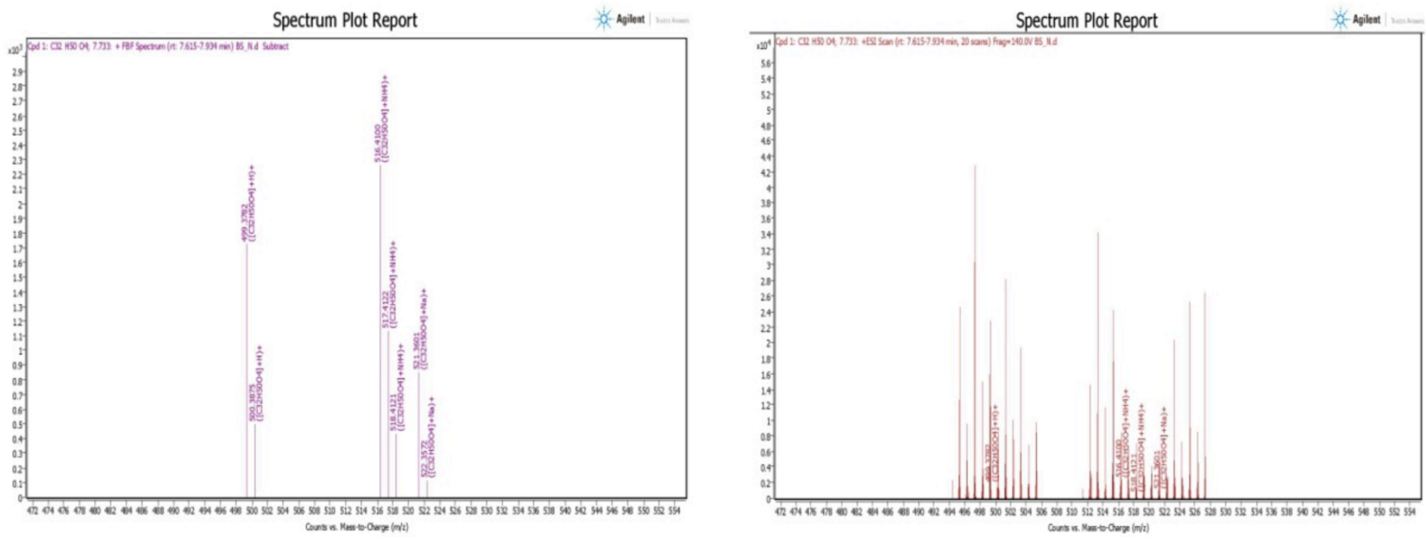


[B]

Figure 2

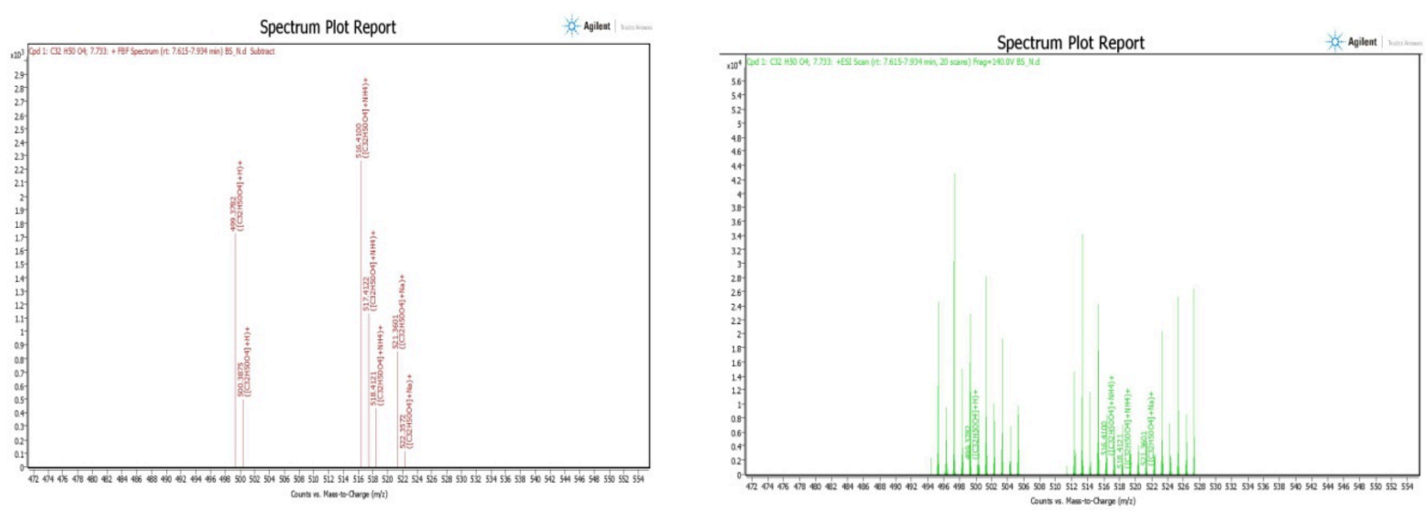
Graph [A] and [B] is the spectrum Plot of AKBA



[A] **[B]**

Figure 3

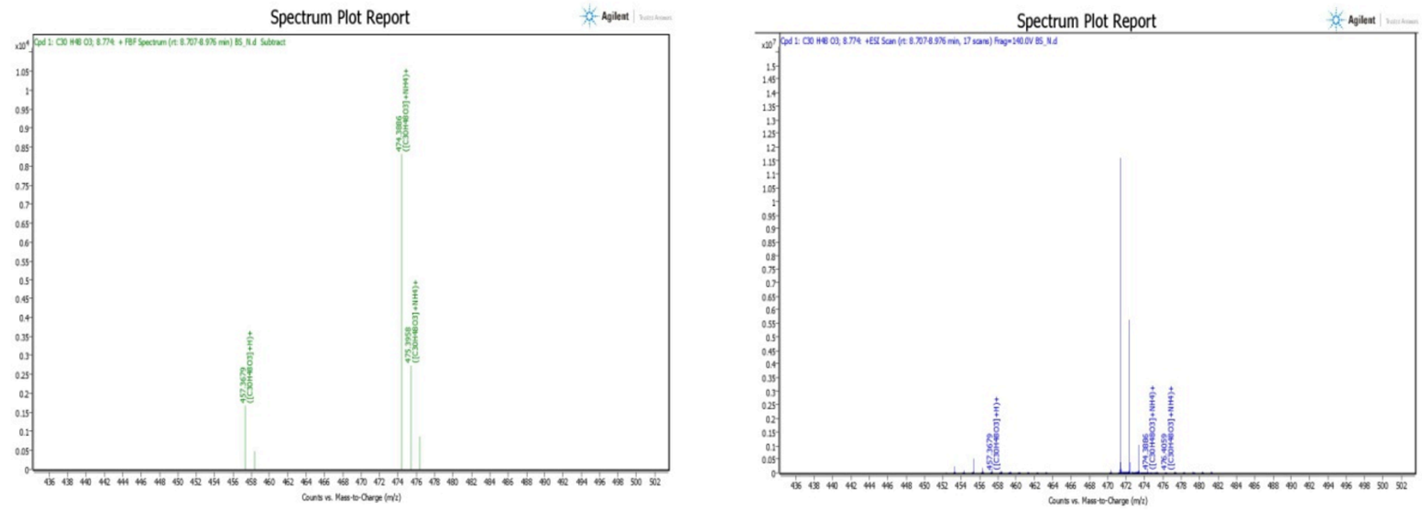
Graph [A] and [B] is the spectrum Plot of α-Boswellic Acid (α-BA)



[A] **[B]**

Figure 4

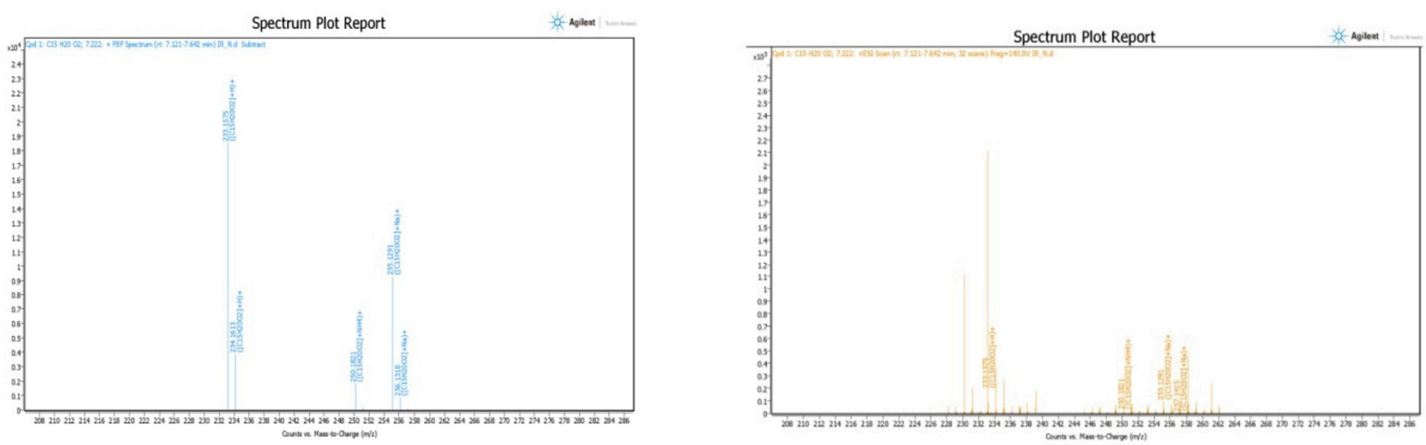
Graph [A] and [B] is the spectrum Plot of Acetyl-β-Boswellic Acid (AB-BA)



[A] [B]

Figure 5

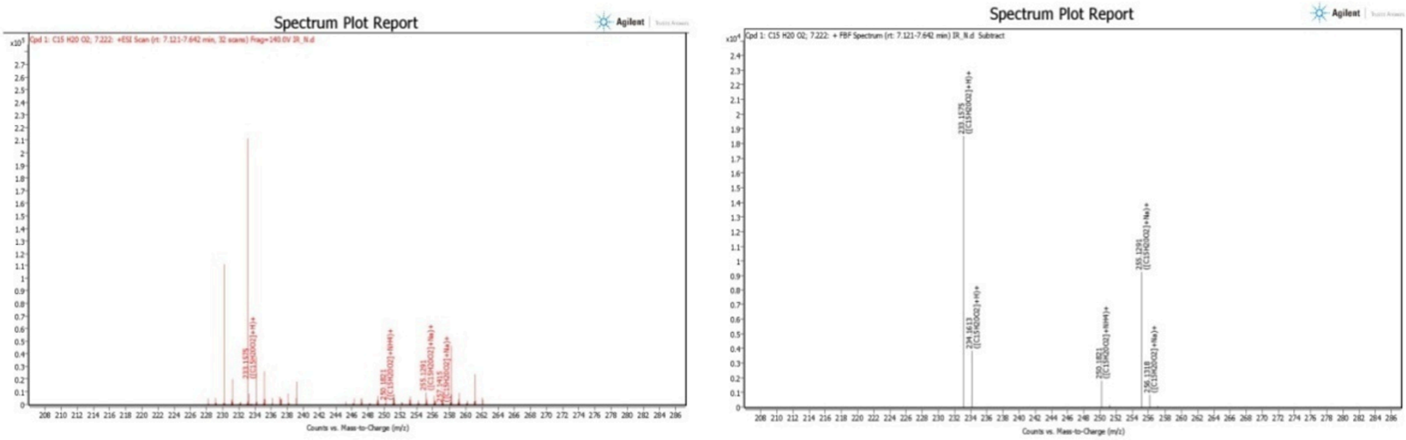
Graph [A] and [B] is the spectrum Plot of β-Boswellic Acid (B-BA)



[A] [B]

Figure 6

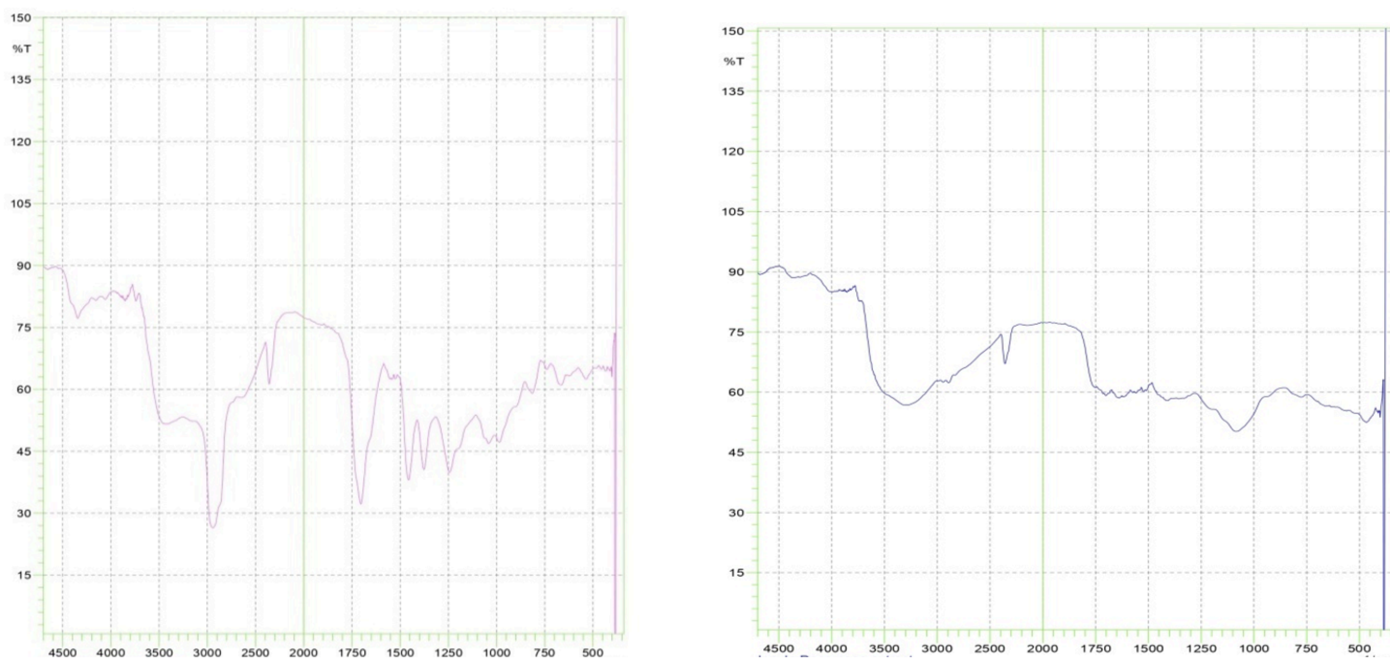
Graph [A] and [B] is the spectrum Plot of Alantolactone



[A] **[B]**

Figure 7

Graph [A] and [B] is the spectrum Plot of Isoalantolactone



[A] **[B]**

Figure 8

Spectral graph of *Boswellia serrata* [A] and *Inula racemosa* [B]

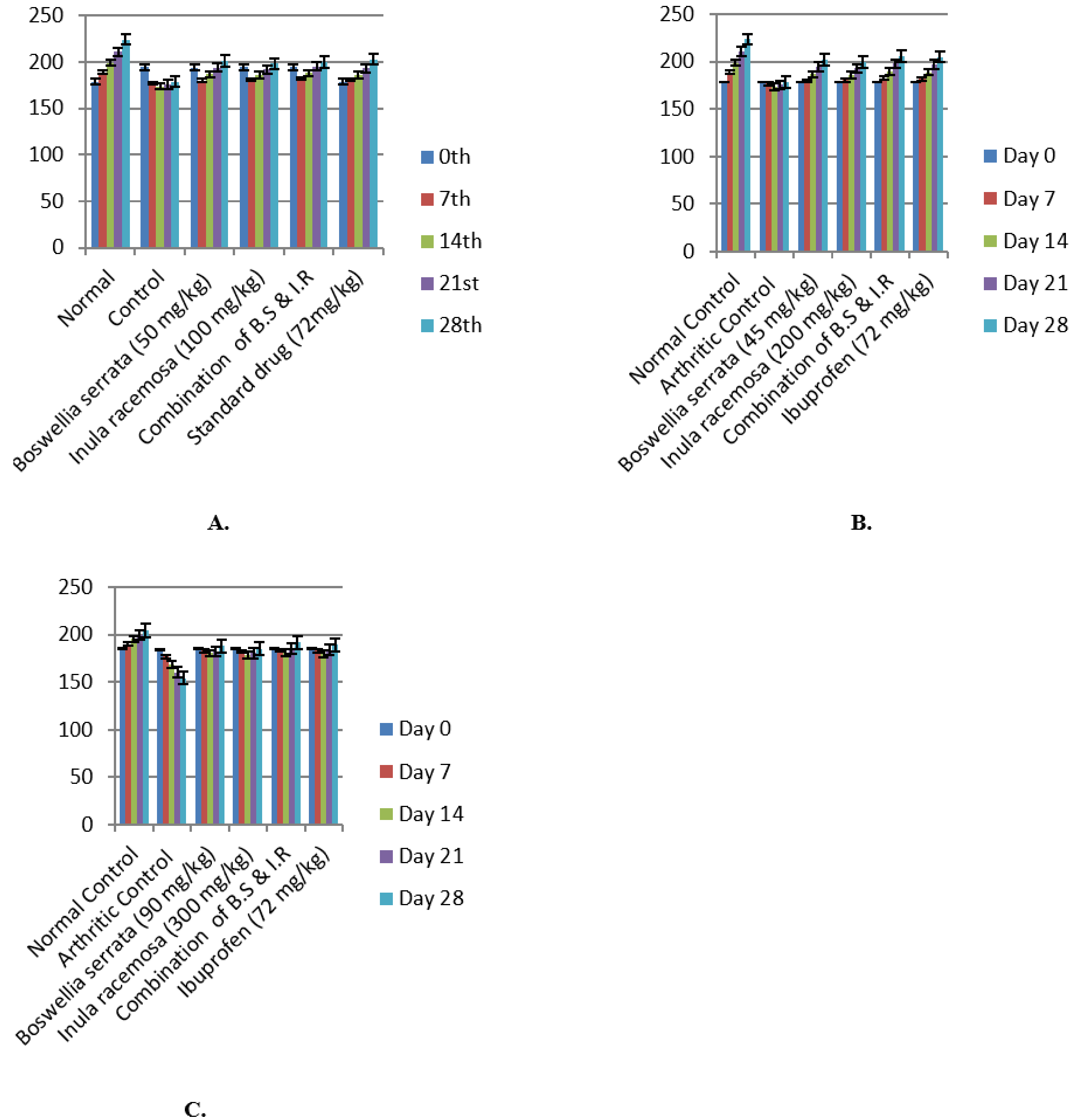
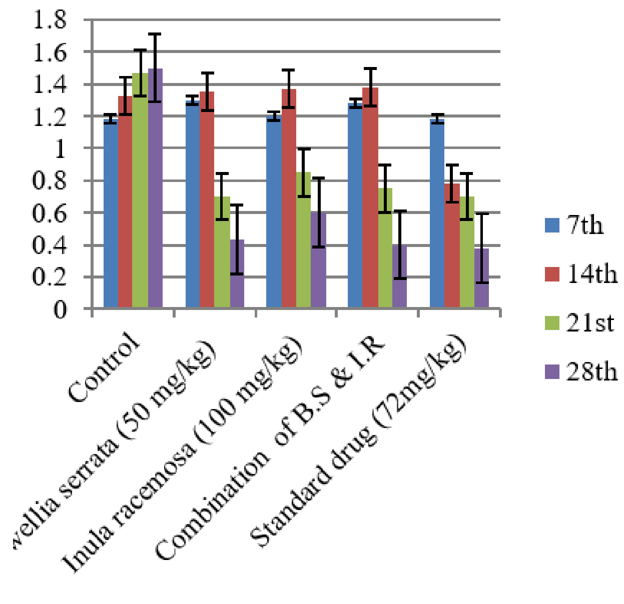


Figure 9

Effect of different treatments on the changes in body weight of rats on different dose levels [A. *Boswellia serrata* (50mg/kg), *Inula Racemosa* (100mg/kg), B. *Boswellia serrata* (45 mg/kg), *Inula Racemosa* (200mg/kg), C. *Boswellia serrata* (90mg/kg), *Inula Racemosa* (300mg/kg) and its combination form].



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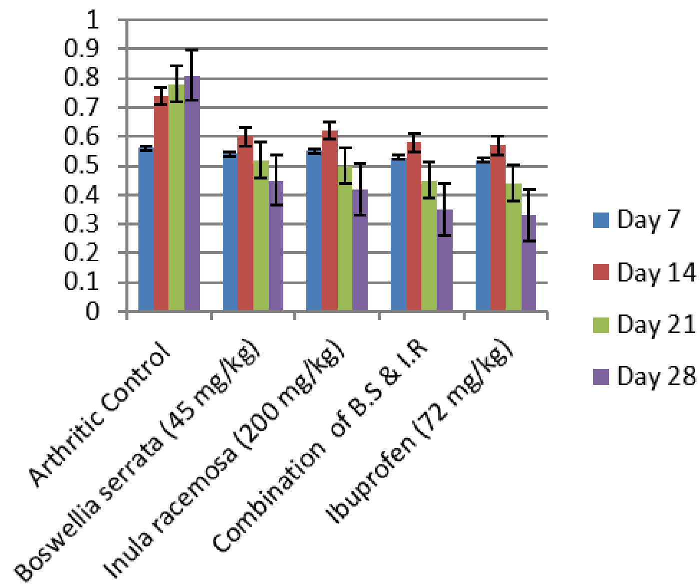

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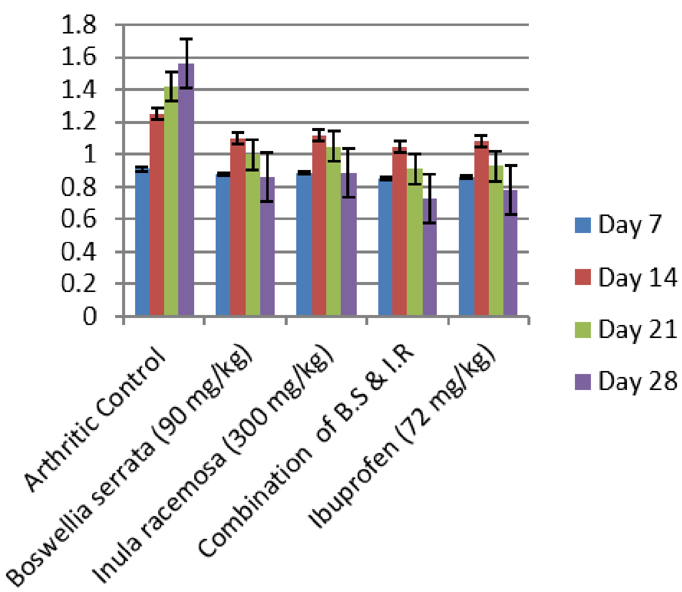


C.

Figure 10

Effect of different treatments on the changes in paw volume of rats on different dose levels [A. *Boswellia serrata* (50mg/kg), *Inula Racemosa* (100mg/kg), B. *Boswellia serrata* (45 mg/kg), *Inula Racemosa* (200mg/kg), C. *Boswellia serrata* (90mg/kg), *Inula Racemosa* (300mg/kg) and its combination form].

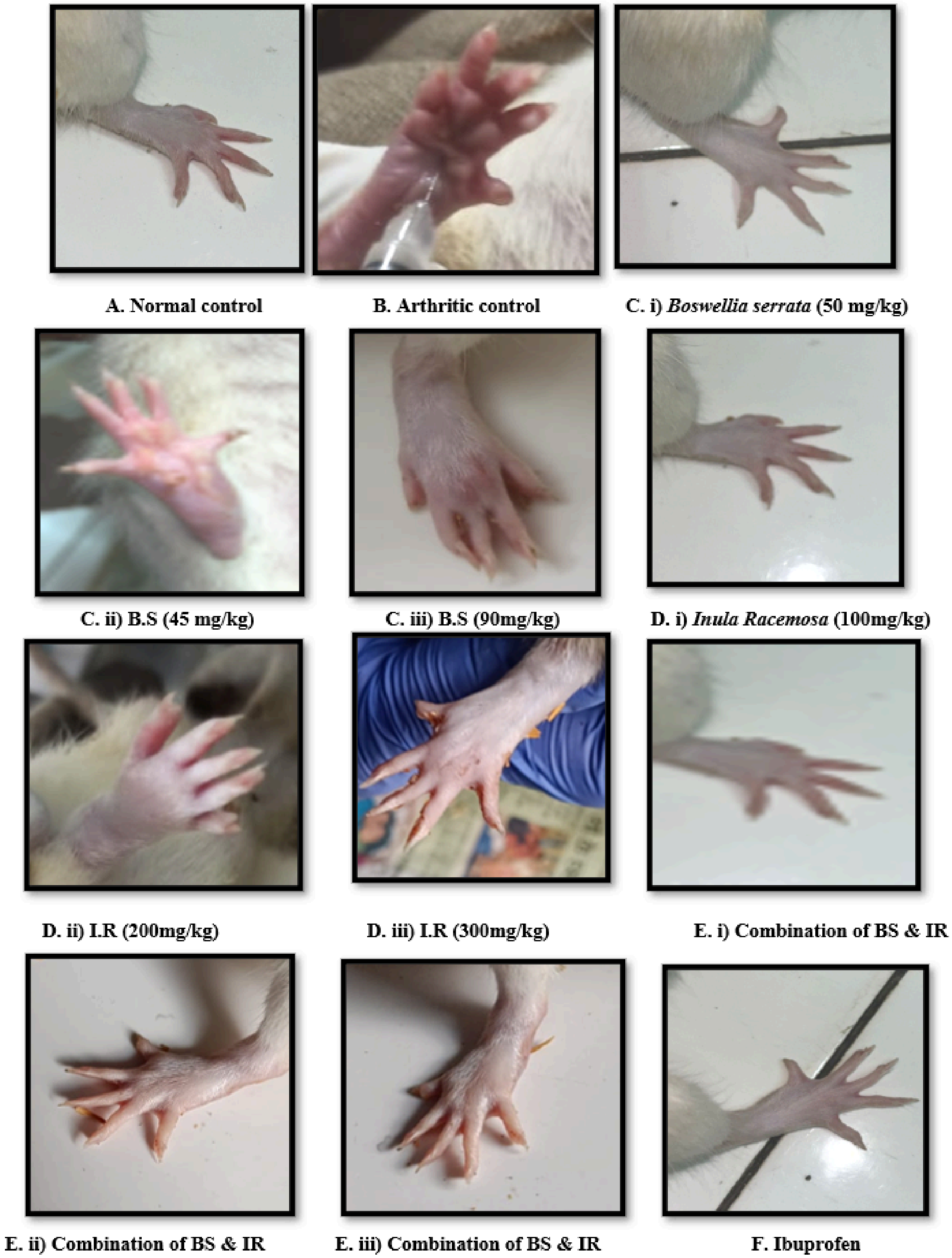


Figure 11

Photographic representations of CFA-induced rat paw edema at 0-day

Figure 12

Photographic representations of CFA-induced rat paw edema at 7th day

Figure 13

Photographic representations of CFA-induced rat paw edema at 14th day

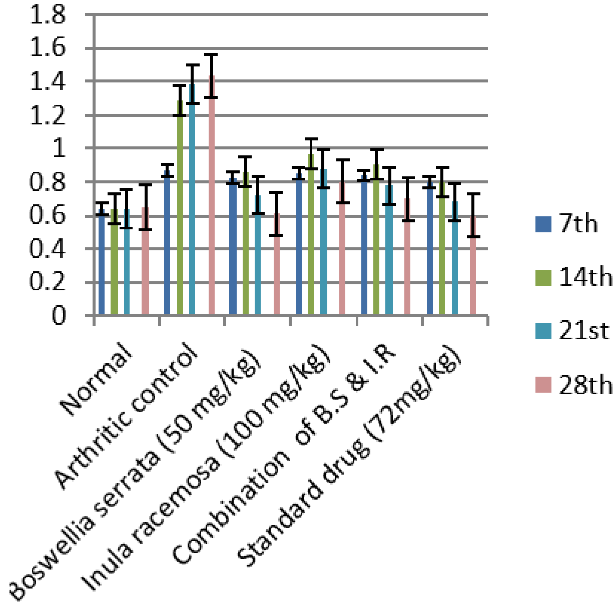
A. Normal group
B. Arthritic control
C. i) *Boswellia serrata* (50 mg/kg)
C. ii) B.S (45 mg/kg)
C. iii) B.S (90 mg/kg)
D. i) *Inula Racemosa* (100mg/kg)
D. ii) I.R (200mg/kg)
D. iii) I.R (300mg/kg)
E. i) Combination of BS & IR
E. ii) Combination of BS & IR
E. iii) Combination of BS & IR
F. Ibuprofen

Figure 14

Photographic representations of CFA-induced rat paw edema at 21st day

Figure 15

Photographic representations of CFA-induced rat paw edema at 28th day



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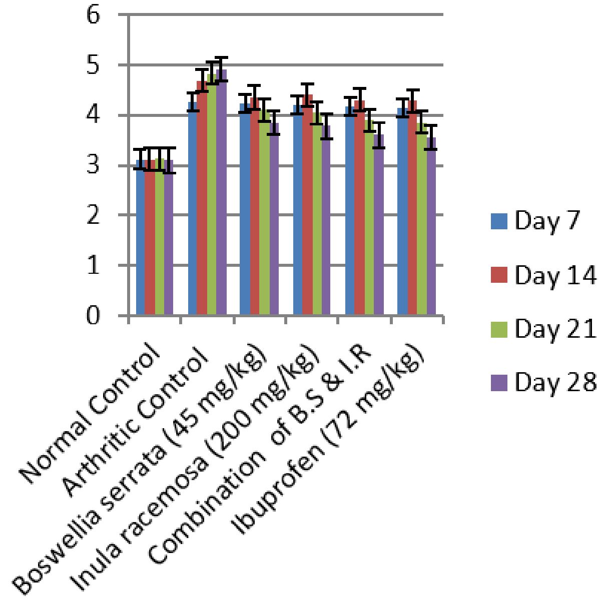

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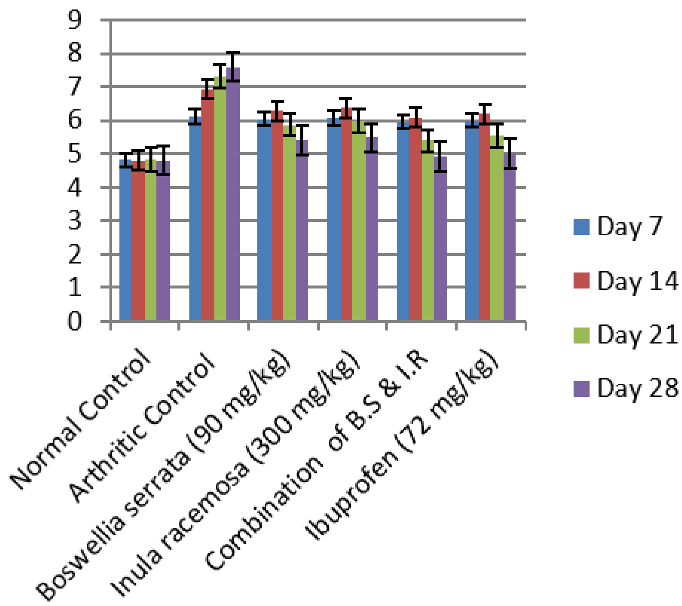
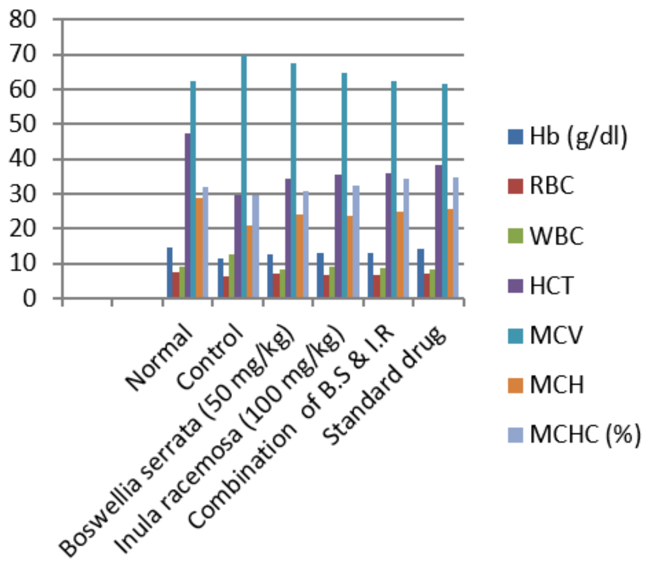


C.

Figure 16

Effect of different treatments on the changes in ankle diameter of rats on different dose levels [A. *Boswellia serrata* (50mg/kg), *Inula Racemosa* (100mg/kg), B. *Boswellia serrata* (45 mg/kg), *Inula Racemosa* (200mg/kg), C. *Boswellia serrata* (90mg/kg), *Inula Racemosa* (300mg/kg) and its combination form].



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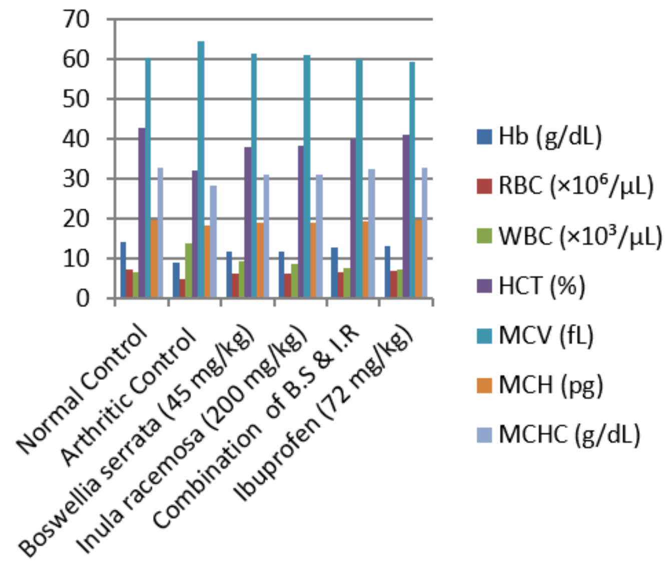

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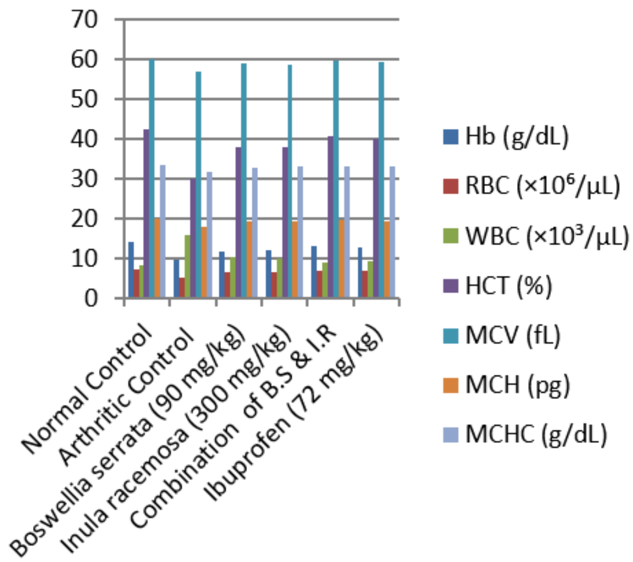
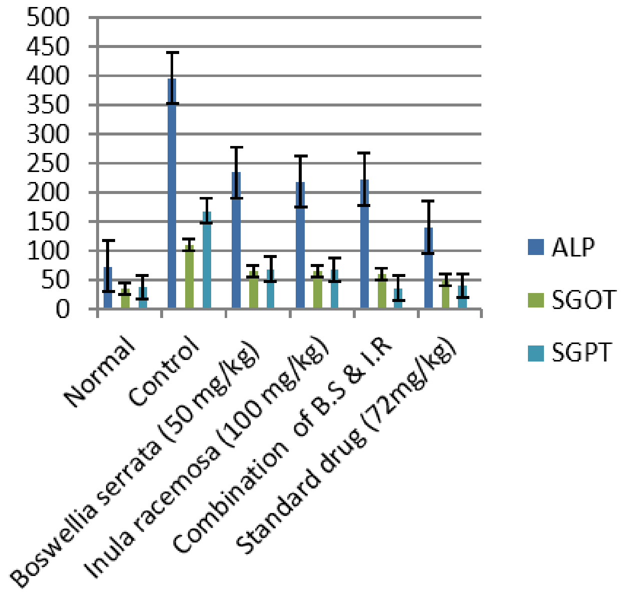


C.

Figure 17

Effect of different treatments on the changes in hematological Parameters of experimental rats on 28th day on different dose levels [A. *Boswellia serrata* (50mg/kg), *Inula Racemosa* (100mg/kg), B. *Boswellia serrata* (45 mg/kg), *Inula Racemosa* (200mg/kg), C. *Boswellia serrata* (90mg/kg), *Inula Racemosa* (300mg/kg) and its combination form].



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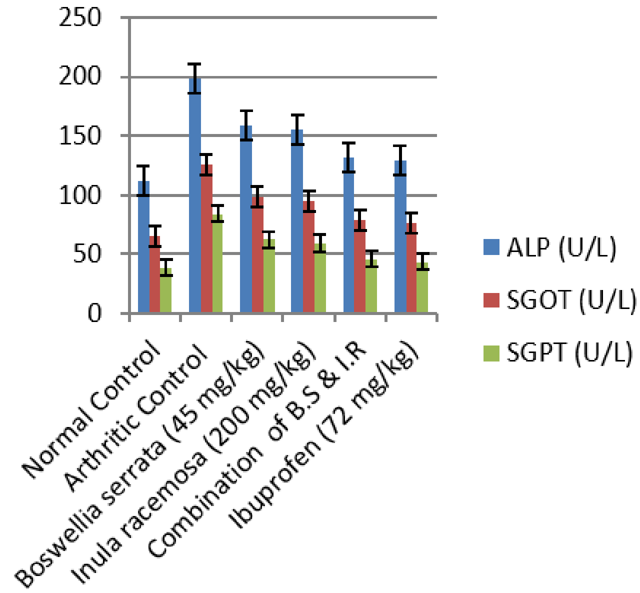

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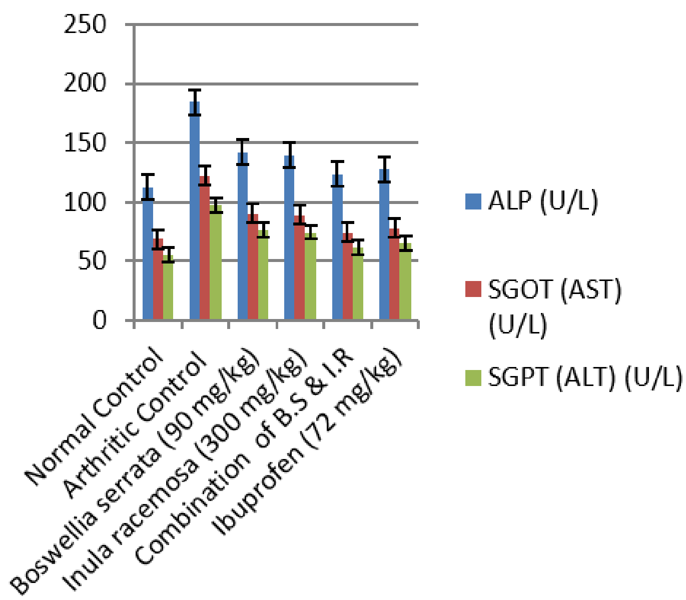

C.

Figure 18

Effect of different treatments on the changes in biochemical parameters of experimental rats on 28th day on different dose levels [A. *Boswellia serrata* (50mg/kg), *Inula Racemosa* (100mg/kg), B. *Boswellia serrata* (45 mg/kg), *Inula Racemosa* (200mg/kg), C. *Boswellia serrata* (90mg/kg), *Inula Racemosa* (300mg/kg) and its combination form].

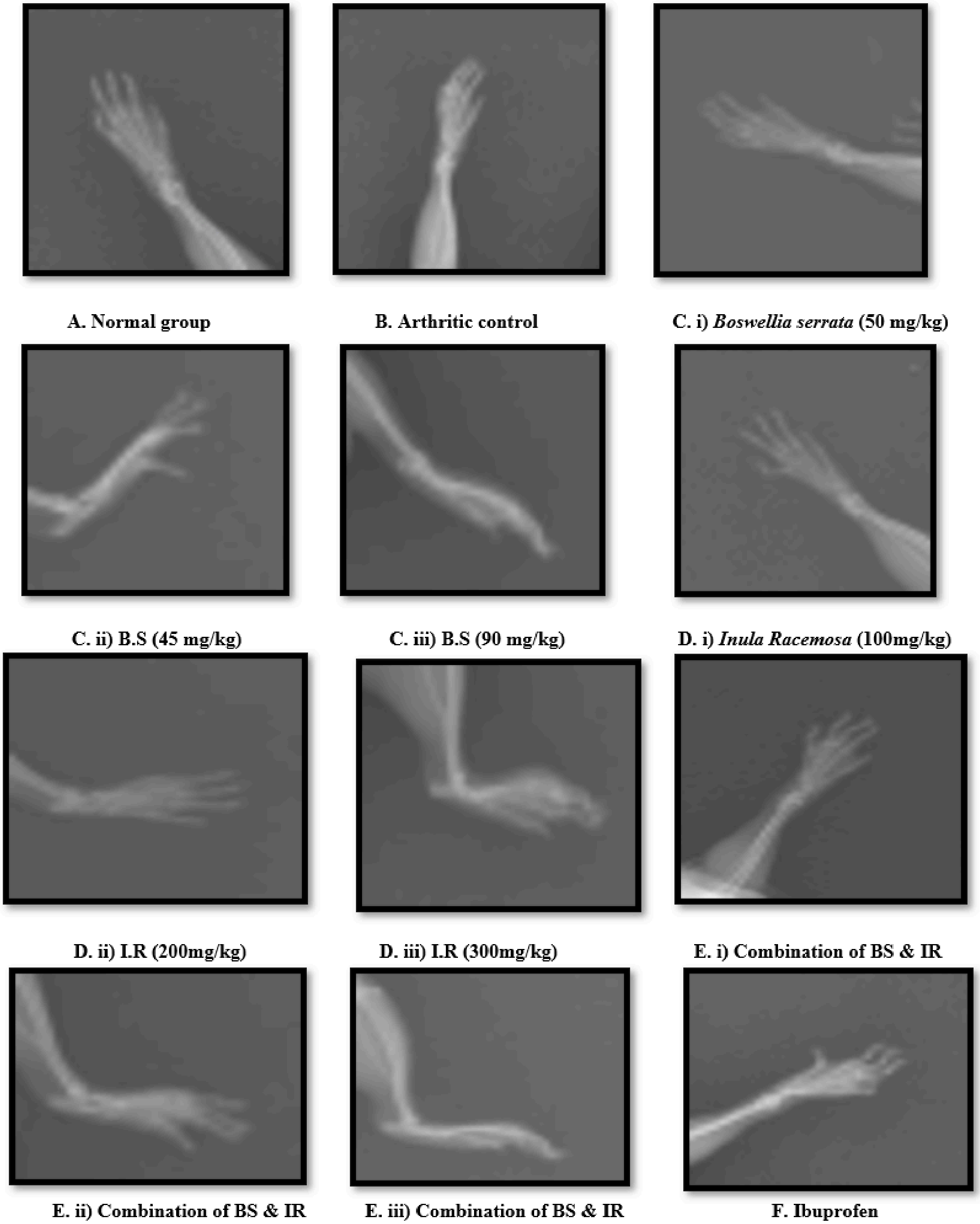

Figure 19

Radiological analysis (x-ray image) of CFA-induced left hind paws of arthritic rats with different treatment groups at day 28th day.

Supplementary Files

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- GA.png