

Anti-inflammatory Activity, Phytochemical Screening and Bioactive Chemical Analysis of *Cicer Arietinum* L. (chickpeas) Using Gas Chromatography-Mass Spectrometry Techniques

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ABSTRACT:

Herbal medicine is the cornerstone of complementary and alternative medicine, which has been progressively gaining global recognition in recent years and moving toward integration into the traditional healthcare system. In both industrialized and developing nations worldwide, the use of herbal medicine transcends ethnic, social, and gender boundaries. A popular legume in the Fabaceae family, chickpeas (*Cicer arietinum* L.) are prized for their nutritional and therapeutic qualities. Chickpeas are a major food legume crop, ranking third in output worldwide after common beans and field peas. They are extensively grown in the subtropical and warm-temperate climates. Its two main varieties, desi and kabuli, differ in terms of phytochemical richness and bioactivity. Preliminary Phytochemical analysis of the Ethanolic extract revealed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids, and phenolic chemicals, indicating possible pharmacological activity. GC-MS research revealed numerous bioactive chemicals, including phytosterols, fatty acid esters, phenolics, and flavonoid derivatives, which have anti-inflammatory, antioxidant, and antibacterial effects. The carrageenan-induced chick paw edema model is used in this work to assess *Cicer arietinum* L. anti-inflammatory properties. In order to produce inflammation, 1% carrageenan was injected subplantarily into seven-day-old chicks after they had received graded dosages of Ethanolic extracts of raw and germinated *C. arietinum* seeds. To assess the anti-inflammatory effect, paw thickness was evaluated at pre-established intervals and compared to a control group and a standard group that received Indomethacin treatment. The *C. arietinum* extract

significantly and dose-dependently decreased paw edema, indicating that it has potent anti-inflammatory qualities.

Keywords: anti-inflammatory, carrageenan-induced chick paw edema, *Cicer arietinum*, GC-MS, Preliminary Phytochemical analysis,

1. INTRODUCTION

Both as a component of conventional healing systems and as an adjunct or substitute for contemporary medical therapies, herbal therapy is still widely used today. Many people are using herbal medicines for a variety of health issues, from simple ailments to chronic diseases, as a result of the growing interest in holistic health and wellness. Since ancient times, people have recognized the therapeutic benefits of traditional herbal remedies. Local medicinal plant knowledge systems are accessible to about 65% of the world's population. India has a wealth of established information about herbal medicine.

1.1. *Cicer arietinum* (Chick peas)

The chickpea plant (*Cicer arietinum*) has various herbal properties, making it a valuable plant in traditional medicine and herbal remedy. They are rich in various chemical constituents that contribute to their nutritional and health benefits. They contain high levels of proteins, making them a valuable source of plant-based protein. Chickpeas are also abundant in dietary fiber, which aids in digestion and promotes GUT health. They contain essential amino acids, vitamins, and minerals like iron, magnesium, and phosphorus. Chickpeas are also rich in phytochemicals like flavonoids, which possess antioxidant properties, digestive aid, anti-inflammatory properties, blood sugar regulation, diuretic properties and support for female health helping to reduce oxidative stress and lower the risk of chronic diseases.

1.2. Anti-inflammatory activity

A medication or other material that lessens the body's inflammation (pain, swelling, and redness). Anti-inflammatory drugs prevent the body from producing some of the chemicals that lead to inflammation. They are employed to treat a wide range of ailments. Natural treatments made from plants that can lower inflammation are known as herbal anti-inflammatory medications.

Based on the aforementioned, the purpose of this study was to evaluate the mechanisms underlying *Cicer arietinum*'s anti-inflammatory action on chicks' paw edema caused by Carrageenan.

2. MATERIALS AND METHODS

2.1. Plant Profile

Cicer arietinum, sometimes known as chickpea, is a leguminous annual herb that grows to a height of less than one metre. It is distinguished by its branching, straight or bending stem, feathery pinnately compound leaves, and little blooms in white, pink, purple, or blue. The plant produces pods with one to four seeds, sometimes known as chickpeas or garbanzo beans.

2.2. Plant collection

The first-graded seeds of the "*Cicer arietinum*" plant are gathered from nearby seed and manure stores, respectively. These seeds are a common variety that is used on a daily basis.

2.3. Preparing a Powder Sample

Black and white *Cicer arietinum* seeds (Desi and Kabuli varieties) were gathered and steeped in water for a full day. After germination in a dark environment, the seeds were dried in the sun and ground into a

coarse powder. For the Desi and Kabuli groups, which did not germinate, the seeds were simply dried and ground into a coarse powder.

2.4. Sample extraction (desi and kabuli chickpeas (Raw and Germination))

The powdered seeds (250 gm) were steeped in 500 ml of ethanol in a 1L beaker with the mouth securely wrapped with foil. The beaker containing ethanol-powered seeds was shaken frequently for one week. After one week, the content was filtered using muslin cloth. The extracted liquid was left in the water bath to evaporate. Finally, leftover content was recovered in a beaker. This method was used to prepare an ethanolic extract of desi and kabuli chickpeas.

2.5. Morphological study

While morphology describes the material from which it is known to occur in a specific shape, morphology is the study of an object's form. Color, taste, shape, size, odor, and other morphological and organoleptic characteristics were noted and assessed botanically.

2.6. Preliminary Phytochemical Screening

Standard procedures were used to perform Phytochemical screening for different phytochemical ingredients in the *Cicer arietinum* Ethanolic extract.

2.7. FT-IR (Fourier Transform Infrared Spectrometer)

Using a Fourier Transform Infrared Spectrometer, the ethanolic extract *Cicer arietinum* was examined within the 4000–400 cm^{-1} range. To identify the presence of functional groups in the extracts, the resulting spectral information was compared to the reference graph.

2.8. GC-MS Analysis of *Cicer arietinum* (Gas Chromatography – Mass Spectrometry)

GC-MS analysis of the Ethanolic extract of *Cicer arietinum* was performed using the equipment Agilent 8890GL Version: 2.6.0.306. The equipment has a MS Capillary Standard non-polar column with dimensions of 30 mm \times 0.25 mm ID \times 0.25 μm film. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was operated at 200° C to 250° C and the oven temperature was programmed as follows: Hold for two minutes at up to 50° C. Cool down to 10° C without holding Hold at 300° C for 10 minutes. The components were identified by comparing their retention indices and using the library's NIST version from 2005. Following a comparison with those found in the computer library connected to the GC-MS instrument, the constituents were identified, and the findings were tallied.

2.9. Anti-inflammatory activity

Inflammation is a complex body response towards the harmful stimuli, i.e., pathogens, damaged cells, irritants. It is involved in tissue repairment. But in chronic form, it has negative effects on the body.

2.9.1. Method:

The anti-inflammatory activity evaluated by using carrageenan-induced paw oedema in chick.

2.9.2. Experimental Animals:

The experimental study was conducted on chicks aged from 7-14 days old and weighting about 35-95g. The chicks will be divided into 4 groups. Each group contains 4 chicks.

2.9.3. Experimental design and drug treatment

2.9.3.1. Animal Preparation & Grouping:

Use seven-day-old chicks and acclimatize them for 24 hours under standard conditions. Divide them into four groups: (1) Positive control (Indomethacin, 25 mg/kg), (2) Experimental Group 1 (DRFE 200 mg/kg), (3) Experimental Group 2 (DRFE 400 mg/kg), (4) Negative control (Normal saline).

2.9.3.2. Carrageenan Injection:

Induce inflammation by injecting 0.1 mL of 1% Carrageenan solution sub-plantar into the right hind paw of each chick.

2.9.3.3. Treatment Administration:

Administer Indomethacin (25 mg/kg), Extract (200 or 400 mg/kg), or Normal saline orally 1 hour after Carrageenan injection, with a maximum volume of 100 mL/kg.

2.9.3.4. Paw Volume Measurement:

Measure the paw volume of each chick using a digital Vernier Caliper before the Carrageenan injection (baseline) and at intervals (e.g., 1, 2, 3, and 4 hours) after injection to assess edema.

2.9.3.5. Data Analysis:

To assess the anti-inflammatory effect, compute the change in paw volume (post-injection volume minus baseline volume) and compare the outcomes between groups. ANOVA is one type of statistical analysis that can be used to identify significant differences.

3. RESULT AND DISCUSSION

Table No: 1 Morphology study of C.arietinum

Characters	Desi (Black) chickpeas	White (Kabuli) chickpeas
Nature of Powder	Coarse powder	Fine powder
Colour	Dark yellow brown	Pale yellow
Taste	Slightly bitter	Slightly sweet
Odour	Aromatic	Unpleasant, Sour smell

Table No: 2 Preliminary Phytochemical Screening of Ethanolic extract of Cicer arietinum

Phyto chemicals	Desi (Raw)	Desi(Germ)	White (Raw)	White(Germ)
Proteins	+	+	+	+
Carbohydrates	+	+	+	+
Flavonoids	-	-	-	-
Phenols and Tannins	-	-	-	-
Saponins	+	+	+	+
Cardiac glycoside	-	+	-	-
Alkaloids	+	+	+	+
Reducing sugar	+	+	+	+
Terpenoids	+	+	+	+
Sterols	+	+	+	+
Fixed oils and fats	+	+	+	+

(+) Presence of constituents (-) Absence of constituents

FTIR - data analysis

Figure: 1 IR Spectrum of Cicer arietinum (germinated black / Desi Chickpeas)

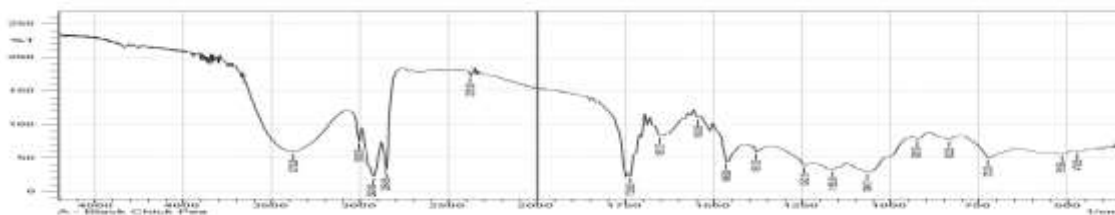


Figure: 2 IR spectrum of Cicer arietinum (germinated white/ white Chickpeas)

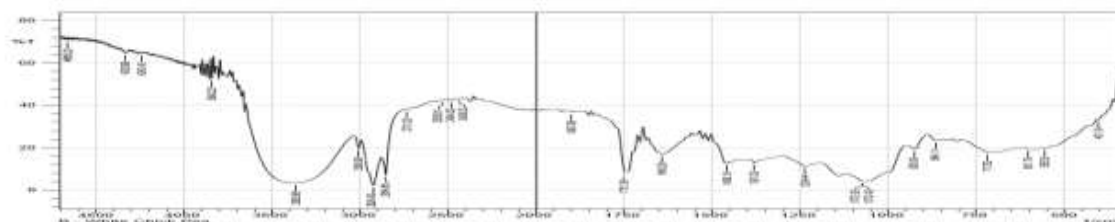


Table No: 3 FTIR-Analysis of Ethanolic extract of Cicer arietinum seeds (germinated black / Desi Chickpeas) showing the functional groups of bio-molecules

Absorption band region (cm ⁻¹)	Functional groups
3379.29	OH-Stretching of alcohol
3008.95	CH of aromatic
2924.09	CH of aliphatic
2854.65	CH of aliphatic
1735.93	C=O lactone
1651.07	Unsaturation C=C ketone
1465.90	Methylene CH ₂
1381.03	Angular methyl group
1242.16	nucleic acid and phospholipids
1165.00	C-O ether linkage (carbohydrate)
1064.71	C-O ether linkage (carbohydrate)

Table No: 4 FTIR-Analysis of Ethanolic extract of Cicer arietinum seeds (germinated white/ white Chickpeas) showing the functional groups of bio-molecules

Absorption band Region (cm ⁻¹)	Functional groups of bio molecule
3363.86	OH-Stretch (protein &carbohydrate)
3008.96	CH of aromatic
2924.09	Aliphatic (due to lipids)
1751.36	Ketone
1643.35	Unsaturation
1458.18	Methyl
1381.03	Angular methyl
1072.42	C-O Ether linkage (carbohydrates)

1072.42	C- O Ether linkage (carbohydrate)
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The bands in chickpeas, which are mostly produced by proteins and carbohydrates, are located between 3423 and 3375.7 cm⁻¹ and indicate O-H and -H stretching vibrations. Lipids are the primary source of C-H stretching vibrations, which are represented by the bands between 2929.8 and 2927 cm⁻¹. The C=O group of the amide-I protein is shown by the bands between 1654.4 and 1652.2 cm⁻¹. The proteins' -CH group was present in the 1456.1–1412.3 cm⁻¹ absorption bands. For nucleic acids and phospholipids, the absorption band between 1245.4 and 1245.1 represents the -P=O phosphodiester groups. The -C-O group from carbohydrates was seen in the 1158.6–1018.3 cm⁻¹ band range.

GC-MS screening report

In the GC-MS studies 19 compounds were identified Ethanolic extract of *Cicer arietinum* (germinated black/ Desi Chickpeas) and 21 compounds in ethanol extract of *Cicer arietinum* (germinated white/white Chickpeas)

Figure: 3 GC-MS of *Cicer arietinum* (germinated black/ Desi Chickpeas)

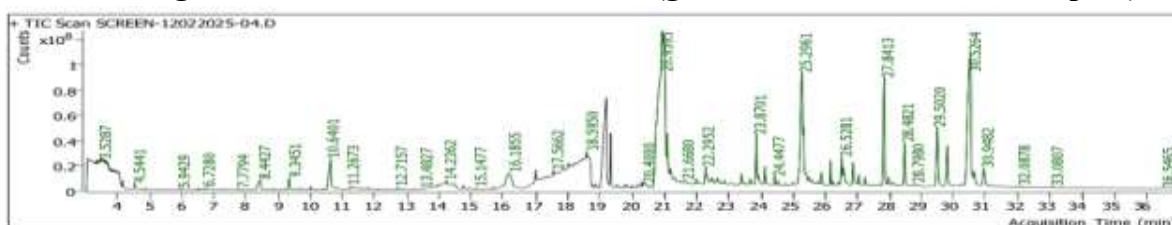


Figure: 4 GC-MS of *Cicer arietinum* (germinated white/white Chickpeas)

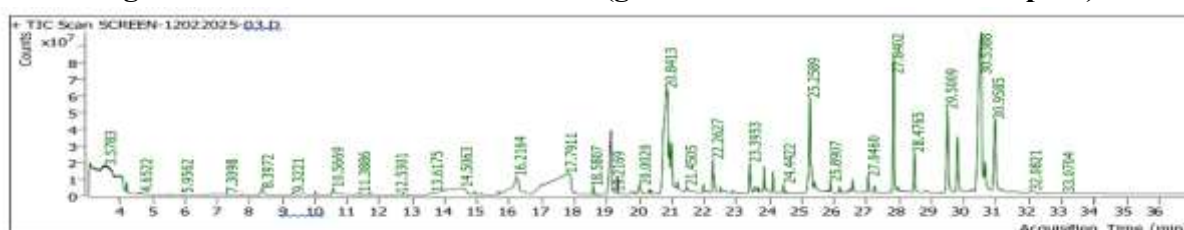


Table No: 5 GC-MS Analysis of Ethanolic of *Cicer arietinum* seeds (germinated Black/ Desi Chickpeas) showing the bioactive constituents

S. No	RT	Compound Name	Mole. Wt	Area%	Uses
01	3.5287	(S)-(+)-1,2-propanediol	76.09g/mol	0.745	Pharmaceutical Industries
02	10.6401	5-Hydroxy methyl furfural	126.11g/mol	1.716	Flavouring agent, Human milk fortifiers.
03	16.1855	D-glucopyranoside	208.09g/mol	1.682	Moisturizing & conditioning effects
04	17.5662	1-Aminoclopentanecarboxylic Acid	325.44g/mol	0.092	Peptide synthesis, Drug development.
05	18.5950	Lidocaine	234.34g/mol	0.141	Local anesthetics
06	20.9595	Linoleic acid	368.44g/mol	15.168	Anti-Inflammatory,
07	23.8701	Hexadecenoic acid	330.50g/mol	1.315	Anti-Hypertensive,

08	25.2961	9,12-Octadecadienoic acid	354.52g/mol	6.018	Anti-Inflammatory,.
09	26.5281	4h-1-Benzopyran-4-one,7-hydro 3-(4-methoxyphenyl)-	268.2641g/mol	0.738	Anti-Inflammatory, Anti-Oxidant.
10	27.8413	Gamma, -Tocopherol	416.68g/mol	3.344	Chronic inflammatory diseases,
11	28.4281	Vitamin E	430.71g/mol	1.338	Anti-Inflammatory, Anti-Oxidant.
12	29.5020	Campesterol	400.68g/mol	2.357	Anti-Inflammatory
13	30.5264	Gamma, -sitosterol	414.706g/mol	10.403	Anti-Inflammatory,
14	8.4427	Clindamycin	424.98g/mol	0.702	Anti –biotic
15	4.236	Butylated Hydroxytoluene	223.35g/mol	0.451	Anti-Oxidant
16	27.0561	Delta. Tocopherol	402.65g/mol	0.255	Anti-Oxidant
18	30.2647	Stigmasterol	412.69g/mol	0.118	Anti-Inflammatory
20	36.5465	Rhodopsin	554.88g/mol	0.031	Photosynthetic bacteria
21	30.9482	beta-Amyrin	426.72g/mol	0.722	Anti-Inflammatory

Table No: 6 GC-MS Analysis of Ethanolic extract of Cicer arietinum seeds (germinated White/ Kabuli Chickpeas) showing the bioactive constituents

S.No	RT	Compound Name	Mole. Wt	Area%	Uses
01	16.21	Ethyl-alpha-d-glucopyranoside	208.09g/mol	2.524	Skin moisturizing property
02	17.79	3-O-Methyl-d-glucose	194.18g/mol	1.806	Blood brain barrier research, Cell culture.
03	20.84	9,12-Octadecadienoic acid	280.45g/mol	9.836	Anti-Oxidant
04	22.26	2-((8Z,11Z)-Heptadeca-8,11- dien-1-yl)-4,5-dihydrooxazole	365.5g/mol	0.886	Anti-Oxidant
05	23.39	2(Dimethylaminoethyl(9Z,12 Z)-octadeca-9,12-dienoate	347.54g/mol	1.139	Gene delivery
06	25.25	Octadecadienoic acid	354.52g/mol	4.267	Anti-Inflammatory
07	27.70	Gamma-Tocopherol	416.68g/mol	0.038	Anti-Inflammatory
08	27.84	Gamma-Tocopherol	416.68g/mol	5.427	Anti-Inflammatory
09	28.47	Vitamin E	430.71g/mol	1.459	Anti-Inflammatory Anti-oxidant.
10	29.50	Campesterol	400.6g/mol	4.296	Anti-Inflammatory, Anti-lipidemic.
11	30.53	. gamma. –Sitosterol	414.70g/mol	16.747	Anti-Inflammatory, Anti-Cancer, Anti-Diabetes, Anti-Oxidant.
12	30.95	. beta. –Amyrin	426.72g/mol	4.142	Anti-Inflammatory,

					Anti-microbial, Anti-oxidant, Anti-Fungal.
13	3.57	Propanoic acid,2-hydroxy-, ethyl ester, (L)-	118.13g/mol	0.816	Cosmetic & food industry
14	8.39	Clindamycin	424.98g/mol	0.493	Anti-bacterial, Anti-biotic.
15	10.56	5-Hydroxymethylfurfural	1126.11g/mol	0.446	Food & pharmaceutical industry.
16	14.50	4H-Pyrazole,3-tert- butylsulfanyl-4,4- bistrifluoromethyl	312.32g/mol	0.777	Physical & chemical properties
17	25.89	Hentriacontane	436.85g/mol	0.230	Anti-Oxidant Carminative Vermifuge
18	26.17	Squalene	410.73g/mol	0.147	Skincare, nutrition, pharmaceutical medicines
19	26.86	Acacetin	284.26g/mol	0.042	Anti-Inflammatory, Anti-Cancer, Anti-diabetic, Anti-obesity.
20	27.04	. delta. –Tocopherol	402.65g/mol	0.434	Anti-Oxidant Vitamin A
21	30.25	Stigmasterol	412.69g/mol	0.167	Anti-Inflammatory, Anti-Cancer, Anti-diabetic, Anti-bacterial,

More than 20 chemicals were found in the ethanol extract of *Cicer arietinum* in the GC-MS investigations. Therefore, the presence of flavonoids, phenols, and saponins in the ethanolic extract may be the cause of *Cicer arietinum*'s current action. The anti-inflammatory action is facilitated by the presence of gamma tocopherol (vitamin E), gamma-sitosterol, and [4H-1-Benzopyran-4-one,5-hydroxy-7-methoxy-3-(4-methoxyphenyl)] biochanin.

Pharmacological study

Figures: 5 Evaluation of The anti-Inflammatory Effect of Ethanolic extract of *Cicer arietinum* in the Carrageenan Induced Inflammation chick Model

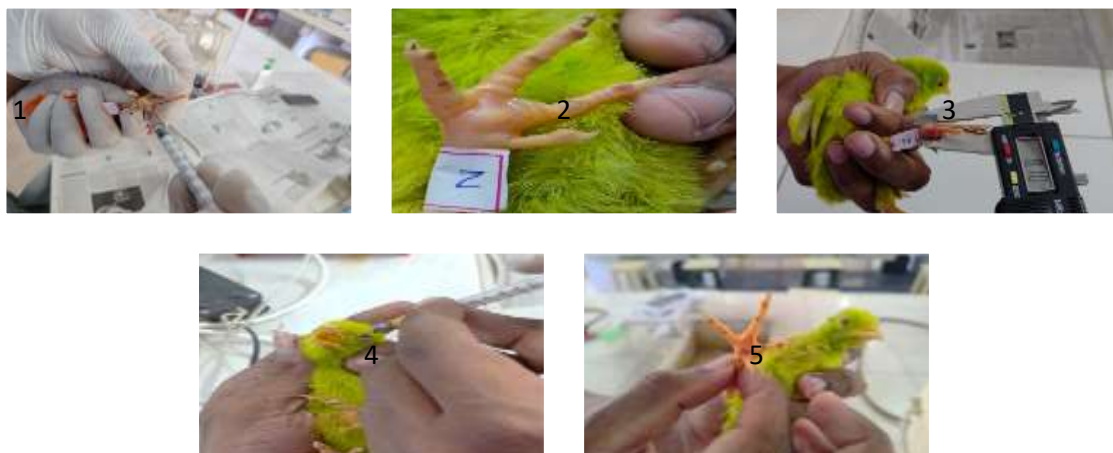
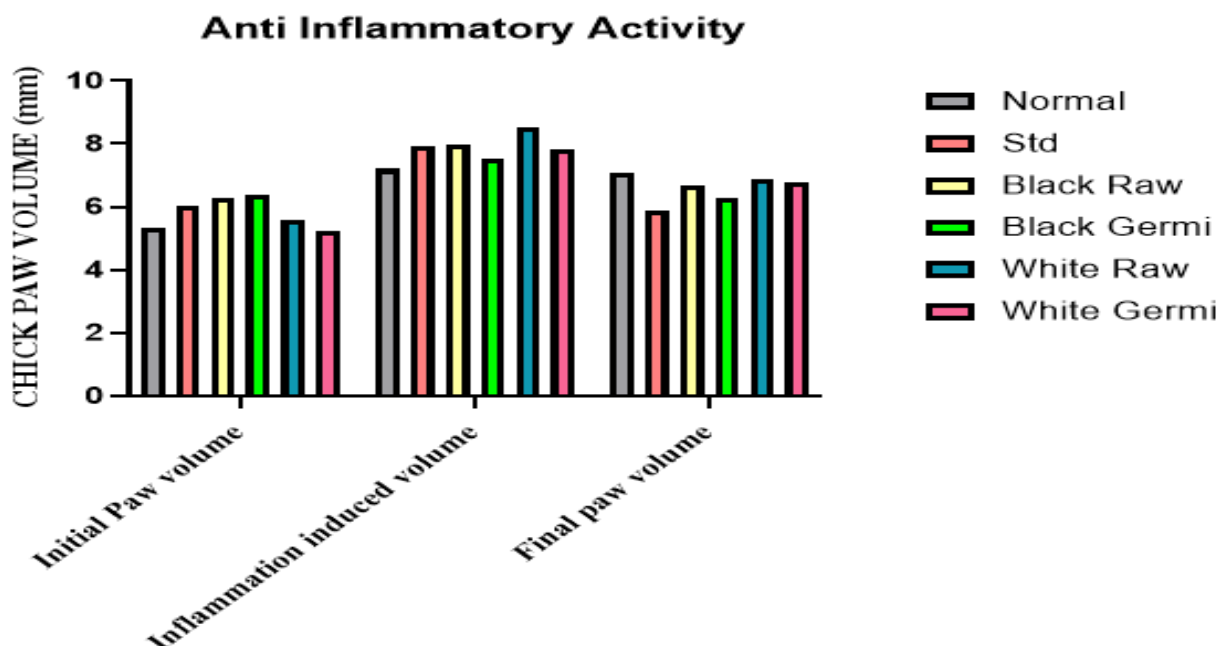


Table No: 6 Percentage Reduction of Paw Volume in Carrageenan induced inflammation

Group	Initial (Before)	Final(After induced Carrageenan)	Paw volume in mm (vernier calliper)			
			1hour	2hour	3hour	4hour
Group I (normal)	5.3±0.25	7.2±0.21	7.3±0.31	7.2±0.35	7.1±0.35	7.10±0.29
Group II (standard)	5.97±0.15	7.9±0.06	7.7±0.06	7.3±0.15	6.6±0.2	5.9±0.1
Group III (test 1)	6.3±0.25	7.96±0.4	8.3±0.55	7.6±0.36	7.3±0.45	6.7±0.35
Group IV (Test 2)	6.4±0.21	7.5±0.45	9±0.36	8.1±0.25	7.3±0.38	6.3±0.3
Group V (Test 3)	4.6±0.06	8.5±0.64	7.96±0.55	7.5±0.55	7.2±0.47	6.9±0.2
Group VI (Test 4)	5.1±0.3	7.8±1.15	7.5±0.3	7.4±0.35	7.1±0.6	6.8±0.64

Figure 6: Effect of DRFE on % inflammation reduction in Carrageenan induced inflammation model on Ethanolic extract of Cicer arietinum



The Ethanolic extract of *Cicer arietinum* showed more significant reduction in paw edema at 3rd hours or more after when injected with Carrageenan, suggesting that *Cicer arietinum* produces an anti-edematous effect during the second phase similar to that of the standard indomethacin.

4. CONCLUSION

The study on *Cicer arietinum* (chickpeas) in both raw and germinated states demonstrated strong anti-inflammatory action, which could be attributable to bioactive chemicals. Preliminary Phytochemical screening confirmed the presence of key secondary metabolites, while GC-MS analysis identified various bioactive constituents, including phenolics, flavonoids, and other bioactive constituents of Vitamin E. Germination increased the concentration of specific bioactive chemicals, potentially boosting medicinal qualities. These findings indicate that germinated chickpeas may be a valuable natural source of anti-inflammatory chemicals, warranting additional research for pharmaceutical and nutraceutical uses.

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