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ANTI- INFLAMMATORY ACTIVITY OF WATER EXTRACT OF *SUNTHI CHOORNA* (POWDER OF DRIED RHIZOME OF *ZINGIBER OFFICINALE* ROSC.) IN LIPOPOLYSACCHARIDE INDUCED CACO-2 CELL MODEL OF INFLAMMATORY BOWEL DISEASE – AN INVITRO STUDY

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ABSTRACT

Background: Inflammatory Bowel Disease (IBD), comprising Crohn's disease and ulcerative colitis, is a chronic inflammatory condition of the gastrointestinal tract characterized by significant morbidity and a complex etiology involving genetic, environmental, and immune factors. Studies shows the prevalence of colorectal carcinoma is also increasing in patients who have a history of IBD. *Sunthi (Zingiber officinale* Rosc.) is one of the reputed drug in *Ayurveda* which can be used as *ahara dravya* and *aushadha* as it is *deepana*, *pachana* and *sangrahi*. Present study aimed to evaluate in vitro anti - inflammatory activity of *Sunthi choorna* (powder of *Zingiber officinale* Rosc.) in Lipopolysaccharide induced Caco-2 cell model of inflammatory bowel disease. **Methodology:** The anti-inflammatory activity were assessed using the assays named Cyclooxygenase activity, Lipoxygenase activity, Myeloperoxidase activity, Inducible nitric oxide synthase, and Estimation of cellular nitrite in lipopolysaccharide induced Caco-2 cell line. **Result and Discussion:** The anti-inflammatory activity has been analysed using anti-inflammatory assays include Cyclooxygenase activity, Lipoxygenase activity, Myeloperoxidase activity, Inducible nitric oxide synthase, and Estimation of cellular nitrite levels and found to be highly significant ($P<0.001$). The in vitro study has been analysed by One way ANOVA with Dunnett multiple comparison test. **Conclusion**: This study

demonstrates the promising anti-inflammatory activity of *Sunthi choorna* (Powder of dried rhizome of *Zingiber officinale* Rosc.) in Lipopolysaccharide-induced Caco-2 cell model of Inflammatory bowel disease.

KEYWORDS: *Zingiber officinale* Rosc., anti-inflammatory activity, Lipopolysaccharide.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory illness affecting the gastrointestinal tract. There are two types: Ulcerative colitis and Crohn's disease. A non-specific inflammatory disease that primarily affects the colon's mucosa and submucosa is known as ulcerative colitis.[1] Regional enteritis is another name for Crohn's disease, in that inflammation can affect any part of the gastrointestinal tract, ranging from the mouth to the anus, but most typically affects the colon and terminal ileum. Acute and chronic inflammatory cells invade the gut wall under both conditions.[2] Exact cause of inflammatory bowel disease is unknown, but there are multiple factors which are considered. Genetic factors, defective immune regulation, exogenous factors including diet stress etc are among them. The main clinical features included in IBD are diarrhea, blood or mucous in stool, abdominal pain and cramping, rectal bleeding, weight loss etc.[3]

Sunthi (Zingiber officinale Rosc.) the highly valuable drug which has been used very commonly in Ayurveda for the diseases related to digestion. Acharya Charaka has mentioned *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) with warm water for *ama pachana* (digests the indigested food) in *grahani chikitsa*.[4] Acharya Susrutha in *Atisara pretisedha adhyaya* has mentioned drugs belongs to *pachana* (aids digestion) *grahi* (water absorbant) and *deepana* (stimulate digestive fire) *gana* along with either *sura* (a fermented beverage), *arishta* (fermented decoctions), *Sneha* (ghee), *mutra* (urine), *sukhambu* (lukewarm water) or *takra* (buttermilk) can be used in the morning.[5]

AIM

Invitro study on anti -inflammatory activity of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in Lipopolysaccharide-induced Caco-2 cell model of inflammatory bowel disease.

OBJECTIVE

1. To assess the anti-inflammatory activity of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in lipopolysaccharide induced Caco-2 cell model of inflammatory bowel disease.

MATERIALS AND METHODS

Preparation of water extract of *Sunthi choorna* (Powder of dried rhizome of *Zingiber officinale* Rosc.) for in vitro study of Anti- inflammatory activity

Water extract of *Sunthi choorna* (Powder of dried rhizome of *Zingiber officinale* Rosc.) was prepared by cold percolation method.50gm *Sunthi choorna* (Powder of dried rhizome of *Zingiber officinale* Rosc.) was added with 500 ml of distilled water and it was kept overnight in a shaker for uniform mixing of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) and distilled water. After that, it was filtered through a muslin cloth. It was dried in a petri plate for 2 days and then scraped the dried extract from the petri plate. The total yield obtained after drying is 32.2g. 1mg sample dissolved in 0.1% Dimethyl sulphoxide and it was kept in Eppendorf having a capacity of 5.0ml. This water extract of *Sunthi choorna* (Powder of dried rhizome of *Zingiber officinale* Rosc.) has been used in the in vitro study.

In vitro cell line study on Anti-inflammatory activity of *Sunthi choorna* (powder of dried rhizome *Zingiber officinale* Rosc.) in Lipopolysaccharide-induced Caco-2 cells

A. Cell culturing

The National Centre for Cell Sciences (NCCS), Pune, India, originally provided the Caco-2 (Human colorectal adenocarcinoma) cell line, which was cultured in Dulbecco's modified Eagles medium (DMEM). The cell line was grown in a 25 cm2 tissue culture flask using Dulbecco's modified Eagle's medium (DMEM), which was enhanced with 10% FBS, L-glutamine, sodium bicarbonate, and an antibiotic solution containing 2.5µg/ml of amphoteracin B, 100µg/ml of streptomycin, and 100U/ml of penicillin. Cultured cell lines were maintained in an incubator with 5% CO2 (carbon dioxide) that was humidified at 37ºC. In addition to preparing chemical stock, cultivated cells were planted in 96-well plates for the study.

i) Cells seeding in 96 well plates

After trypsinizing a confluent monolayer of cells that was two days old, the cells were suspended in 10% growth media. A 96-well tissue culture plate was seeded with 100µl of the cell suspension (5x10 3 cells/well), and the plate was then incubated at 37ºC in a humidified

5% CO2 (carbon dioxide) incubator.

ii) Preparation of compound stock

Using a cyclomixer, a 1 mg sample was weighed and dissolved in 1 ml of 0.1% dimethyl sulphoxide (DMSO). To guarantee sterility, the sample solution was filtered using a 0.22 µm Millipore syringe filter.

B. Anti-inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.)

a) Aim

To determine the anti- inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in lipopolysaccharide (LPS) induced Caco-2 cell lines.

b) Procedure

After the cells reached 60% confluency, 1 µL of lipopolysaccharide (LPS: 1µg/mL) was added to activate them. Caco-2 cells that were stimulated by lipopolysaccharide were subjected to varying concentrations of sample solution (12.5 µg/mL, 25 µg/mL, and 50 µg/mL) and allowed to incubate for a full day. The cell lysate was used for the anti-inflammatory tests following incubation. Cyclooxygenase activity, Lipoxygenase activity, Myeloperoxidase activity, Inducible Nitric Oxide Synthase, and Estimation of Cellular Nitrite Levels are the anti-inflammatory assays.

i) Cyclooxygenase (COX) activity

a) Procedure

The Walker and Gierse method was utilized to measure the Cyclooxygenase (COX) activity. For one minute at 25°C, 100µl of cell lysate was treated with Tris-HCl buffer (pH 8), glutathione at 5 mM/L, and hemoglobin at 5 mM/L. 200 mM/L of arachidonic acid was added to start the reaction, and 200µL of 10% trichloroacetic acid in 1 N hydrochloric acid was added to end it after 20 minutes of incubation at 37°C. The tubes were heated for 20 minutes following centrifugal separation and the addition of 200µL of 1% thiobarbiturate. The tubes were centrifuged for three minutes after cooling. The absorbance at 632 nm was used to measure the activity of cyclooxygenase (COX).

b) Calculation

Percentage inhibition of the enzyme was calculated as,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

ii) Lipoxygenase (LOX) activity

a) Procedure

To measure the activity of lipoxygenase (LOX), 200 µL of sodium linoleate was added to the reaction mixture (2 mL final volume) along with 50 µL of cell lysate and Tris-HCl buffer (pH 7.4). The production of 5-hydroxyeicosatetraenoic acid is reflected by a rise in absorbance at 234 nm, which is used to measure the lipoxygenase (LOX) activity.

b) Calculation

Percentage inhibition of the enzyme was calculated as,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

iii) Myeloperoxidase (MPO) activity

a) Procedure

The cell lysate was homogenized in a solution containing 0.57% hexadecyl trimethyl ammonium bromide (HTAB) and 50 mM potassium phosphate buffer. The samples were then centrifuged at 2000 g for 30 minutes at 4°C, and the activity of myeloperoxidase (MPO) was measured in the supernatant. The addition of 50 mM phosphate buffer (pH 6) containing 1.67 mg/mL guaiacol and 0.0005% H2O activated the sample's myeloperoxidase (MPO). At 460 nm, the absorbance changed and was measured. The activity of myeloperoxidase (MPO) was expressed in units per milliliter of cell lysate. The degradation of 1 µM of peroxide per minute at 25°C was defined as one unit of myeloperoxidase (MPO) activity.

b) Calculation

Enzyme activity was calculated as,

$$U = \frac{\Delta OD \cdot 4 \cdot Vt \cdot \text{dilution factor}}{(L \cdot £470 \cdot \Delta t \cdot Vs)}$$

ΔOD = density change

Vt = total volume (mL) (1.1 mL)

L=light path (1 cm)

£470 = extinction coefficient for tetraguaiacol (26.6 mM-1·cm-1,)

iv) Inducible Nitric Oxide Synthase

a) Procedure

Two ml of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer were used to homogenize the cell lysate. 0.1 ml -2µmol/L L-arginine, 0.1 ml-4µmol/L manganese chloride, 0.1 ml-10µmol/L 30µg dithiothreitol (DTT), 0.1 ml-1 mmol/L NADPH (reduced nicotinamide adenine dinucleotide phosphate), 0.1 ml-4µmol/L tetrahydropterin, 0.1 ml 10µmol/L oxygenated haemoglobin, and 0.1 ml enzyme were added to the assay system. At 401 nm, an increase in absorbance was seen.

b) Calculation

Percentage inhibition of the enzyme was calculated as,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

v) Estimation of Cellular Nitrite Levels

a) Procedure

0.1 mL of 3% sulphosalicylic acid was added to 0.5 mL of cell lysate, and the mixture was vigorously vortexed for 30 minutes. After that, the samples were centrifuged for 15 minutes at 5,000 rpm. The amount of nitrite was estimated using the protein-free supernatant. After adding 30µL of 10% NaOH and 300µL of Tris-HCl buffer to 200µL of the supernatant, thoroughly mix the mixture. Following a dark incubation period of 10–15 minutes, 530µL of Griess reagent containing 1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthyl ethylene diaminedihydrochloride was added. The absorbance was then measured at 540 nm in comparison to a Griess reagent blank. The standard was a solution of sodium nitrite. The standard curves were used to estimate the concentration of nitrite in the samples.

RESULTS

A. Anti-inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.)

Anti-inflammatory activity can be done by anti-inflammatory assays including Cyclooxygenase (COX) activity, Lipoxygenase (LOX) activity, Myeloperoxidase (MPO) activity, Inducible nitric oxide synthase, Estimation of cellular nitrite levels**.** Sample concentrations of 12.5 µg/mL, 25 µg/mL and 50 µg/mL were used to analyse the anti-

inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) and the observations were tabulated.

i) Cyclooxygenase (COX) activity

Cyclooxygenase activity has been conducted and the percentage of inhibition of activity has been noted. With the increase in sample concentration, the percentage of inhibition of cyclooxygenase activity was also increasing. When the sample concentration was 12.5 µg/mL the percentage of inhibition of cyclooxygenase activity was 12.723%, at 25 µg/mL it was 33.841% and at 50 µg/mL it was 49.238%. The values have been tabulated as follows.

Table 1: Cyclooxygenase activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in different sample concentrations.

Sample Concentration (µg/mL)	Mean of percentage of inhibition (%)
Control (Lipopolysaccharide-induced Caco-2 cells)	0
12.5	12.72387
25	33.84122
50	49.23817

ii) Lipoxygenase (LOX) activity

In Lipoxygenase (LOX) activity with the increase of sample concentration, the percentage of inhibition of activity was also increasing. When the sample concentration was 12.5 µg/mL the percentage of inhibition of was 11.002%, at 25 µg/mL it was 37.95 % and at 50 µg/mL it was 49.494%. The values have been tabulated as follows.

Table 2: Lipoxygenase activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in different sample concentrations.

Sample Concentration (µg/mL)	Mean of percentage of inhibition (%)
Control (Lipopolysaccharide-induced Caco-2 cells)	0
12.5	11.00289
25	37.95094
50	49.49495

iii) Myeloperoxidase (MPO) activity

In Myeloperoxidase (MPO) activity with the increase of sample concentration, the enzyme activity was reducing. While inducing with LPS the enzymic activity was 0.0199 U/ml, while the sample concentration was 12.5 µg/mL the of enzyme activity was 0.0165 U/ml, at 25

µg/mL it was 0.0133 U/ml and at 50 µg/mL it was 0.0075 U/ml. The values have been tabulated below.

Table 3: Myeloperoxidase activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in different sample concentrations.

Sample Concentration (µg/mL)	Mean of Enzyme activity (U/ml)
Control (Lipopolysaccharide-induced Caco-2 cells)	0.019954
12.5	0.0165
25	0.013332
50	0.007535

iv) Inducible Nitric Oxide Synthase

In Inducible nitric oxide synthase, with the increase of sample concentration, the percentage of inhibition of activity was also increasing. When the sample concentration was 12.5 µg/mL the percentage of inhibition of was 12.72%, at 25 µg/mL it was 33.841% and at 50 µg/mL it was 49.238%. The values have been tabulated as follows.

Table 4: Inducible Nitric Oxide Synthase of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in different sample concentrations.

Sample Concentration (µg/mL)	Mean of percentage of inhibition (%)
Control (Lipopolysaccharide-induced Caco-2 cells)	0
12.5	12.72387
25	33.84122
50	49.23817

v) Estimation of Cellular Nitrite Levels

In the Estimation of Cellular Nitrite Levels activity with the increase of sample concentration, the concentration of nitrite was reducing. While inducing with LPS the concentration of nitrite was 499.12 µg, while the sample concentration was 12.5 µg/mL the concentration of nitrite was 406.89 µg, at 25 µg/mL it was 350.46 µg and at 50 µg/mL it was 284.29 µg. The values have been tabulated below.

Table 5: Estimation of Cellular Nitrite Levels of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in different sample concentrations.

Sample Concentration (µg/mL)	Mean of concentration of nitrite (µg)
Control (Lipopolysaccharide-induced Caco-2 cells)	499.125
12.5	406.89
25	350.46
50	284.295

STATISTICAL ANALYSIS

The Anti-inflammatory activity of the different concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL) of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) in lipopolysaccharide induced Caco-2 cells was compared to that of the control cells (lipopolysaccharide induced Caco-2 cells). The anti-inflammatory assays including Cyclooxegenase (COX) activity, Lipoxygenase (LOX) activity, Myeloperoxidase (MPO) activity, Inducible nitric oxide synthase, and Estimation of cellular nitrite level have been analyzed. All experiments were done in triplicates, representing the results as Mean ± SE. One-way ANOVA with Dunnett's multiple comparison tests was used to analyze the significance between the control and the various concentrations of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.).

i.Cyclooxygenase (COX) activity

The anti-inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) has done on 3 different concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL) by Cyclooxygenase (COX) activity and found highly significant as shown in the table.

Table 6: Comparison of anti-inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc) of different concentrations by analyzing Cyclooxygenase (COX) activity with One-way ANOVA test.

Variation	S.S	D.F	M.S.S	F value	P < 0.05	Summary
Between samples	4311	3	1437	1172	Yes	****

Table 7: Comparison of anti-inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc) of different concentrations by analyzing Cyclooxygenase (COX) activity with Dunnett's multiple comparison test.

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Control vs 12.5µg/mL	-12.72	14.08	Yes	****	-15.33 to -10.12
Control vs 25µg/mL	-33.84	37.44	Yes	****	-36.44 to -31.24
Control vs 50µg/mL	-49.24	54.47	Yes	****	-51.84 to -46.64

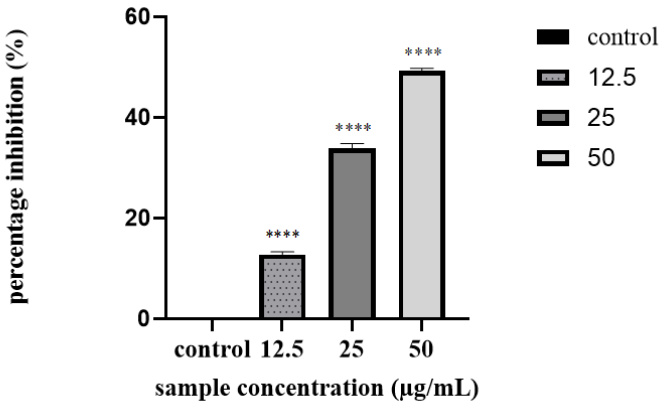

Diagram1: Graphical representation illustrating the effect of the water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) on Cyclooxygenase (COX) activity. Each data represents the Mean ± SE of three replicates.

ii. Lipoxygenase (LOX) activity

The anti- inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) by Lipoxygenase (LOX) activity has been done on 3 different concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL). The anti-inflammatory activity by inhibiting Lipoxygenase (LOX) activity at the sample concentrations 12.5 µg/mL was found to be moderately significant and at sample concentrations 25 µg/mL and 50 µg/mL were found highly significant as shown in the table.

Table 8: Comparison of anti-inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) of different concentrations by analyzing Lipoxygenase (LOX) activity with One-way ANOVA test.

Variation	S.S	D.F	M.S.S	F value	P < 0.05	Summary
Between samples	4764	3	1588	286.8	Yes	****

Table 9: Comparison of anti-inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) of different concentrations by analyzing Lipoxygenase (LOX) activity with Dunnett's multiple comparison test.

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Control vs 12.5µg/mL	-11.00	5.727	Yes	**	-16.54 to -5.470
Control vs 25µg/mL	-37.95	19.75	Yes	****	-43.48 to -32.42
Control vs 50µg/mL	-49.49	25.76	Yes	****	-55.03 to -43.96

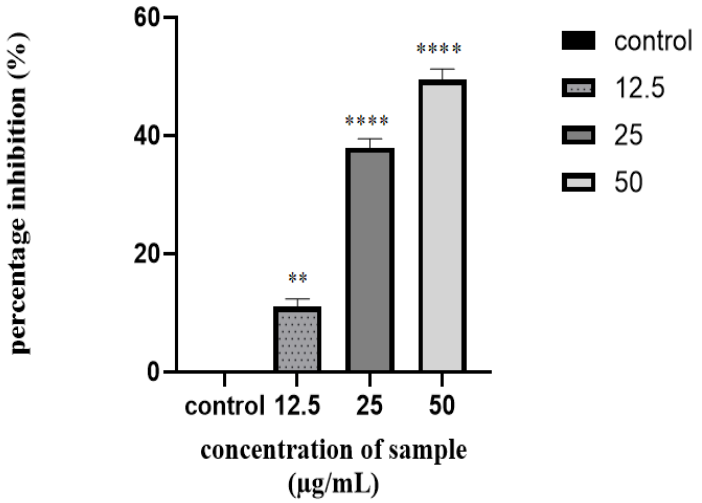

Diagram 2: Graphical representation illustrating the effect of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) on Lipoxygenase (LOX) activity. Each data represents the Mean ± SE of three replicates.

iii. Myeloperoxidase (MPO) activity

The anti- inflammatory activity of water extract of *Sunthi choorna* (powder dried rhizome of *Zingiber officinale* Rosc.) by Myeloperoxidase (MPO) activity has done on 3 different concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL). The enzyme activity has been analyzed and found to be highly significant in all the 3 concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL) of sample and the data shown in the table.

Table 10: Comparison of anti- inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) of different concentrations by analyzing Myeloperoxidase activity with One-way ANOVA test.

Variation	S.S	D.F	M.S.S	F value	P < 0.05	Summary
Between samples	0.0002505	3	8.351	277.2	Yes	****

Table 11: Comparison of anti- inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) of different concentrations by analyzing Myeloperoxidase activity with Dunnett's multiple comparison test.

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Control vs 12.5µg/mL	0.003454	7.707	Yes	***	0.002163 to 0.004745
Control vs 25µg/mL	0.006622	14.78	Yes	****	0.005331 to 0.007913
Control vs 50µg/mL	0.01242	27.71	Yes	****	0.01113 to 0.01371

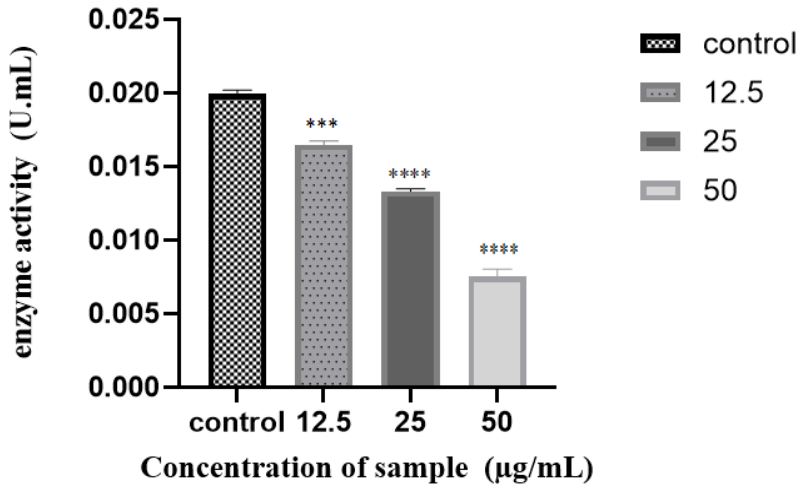

Diagram 3: Graphical representation illustrating the effect of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) on Myeloperoxidase activity. Each data represents the Mean ± SE of three replicates.

iv. Inducible nitric oxide synthase

The anti- inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) by Inducible nitric oxide synthase was done on 3 different concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL) and found to be very significant in all 3 different concentrations. Data was shown in the table below.

Table 12: Comparison of anti- inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) of different concentrations by analyzing Inducible nitric oxide synthase with One-way ANOVA test.

Variation	S.S	D.F	M.S.S	F value	P < 0.05	Summary
Between samples	4307	3	1436	797	Yes	****

Table 13: Comparison of anti-inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) of different concentrations by analyzing Inducible nitric oxide synthase with Dunnett's multiple comparison test.

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Control vs 12.5µg/mL	-12.70	11.59	Yes	****	-15.86 to -9.545
Control vs 25µg/mL	-33.81	30.85	Yes	****	-36.96 to -30.65
Control vs 50µg/mL	-49.22	44.91	Yes	****	-52.37 to -46.06

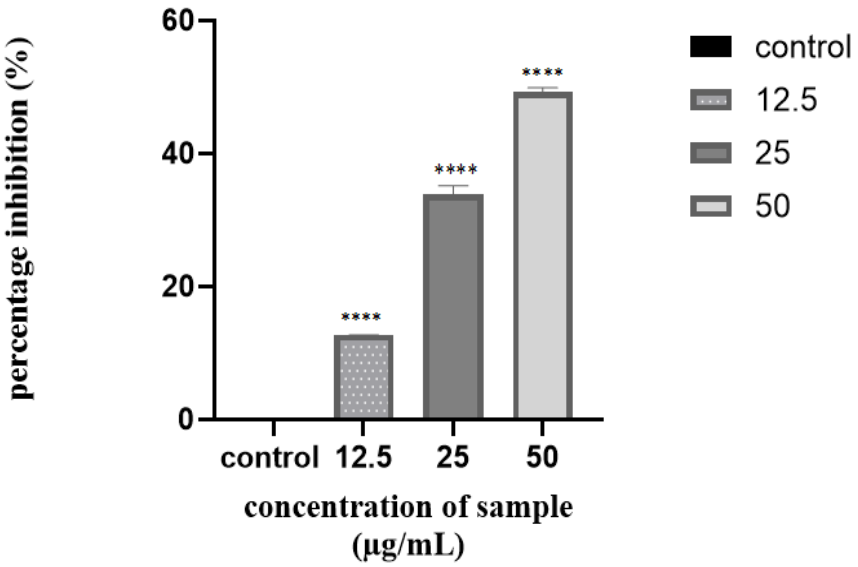

Diagram 4: Graphical representation illustrating the effect of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) on Inducible nitric oxide synthase. Each data represents the Mean ± SE of three replicates.

v. Cellular nitrite level

The anti- inflammatory activity of water extract of *Sunthi choorna* (powder of *Zingiber officinale* Rosc.) by assessing the cellular nitrite level of 3 different concentrations of the sample (12.5 µg/mL, 25 µg/mL , 50 µg/mL) and has been found very significant in the 3 different concentrations of sample (12.5 µg/mL, 25 µg/mL , 50 µg/mL).

Table 14: Comparison of anti- inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) of different concentrations by analyzing cellular nitrite level with One-way ANOVA test.

Variation	S.S	D.F	M.S.S	F value	P < 0.05	Summary
Between samples	74514	3	24838	102.8	Yes	****

Table 15: Comparison of anti- inflammatory activity of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) of different concentrations by analyzing cellular nitrite level with Dunnett's multiple comparison test.

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Control vs 12.5µg/mL	92.24	7.266	Yes	***	55.68 to 128.8
Control vs 25µg/mL	148.7	11.71	Yes	****	112.1 to 185.2
Control vs 50µg/mL	214.8	16.92	Yes	****	178.3 to 251.4

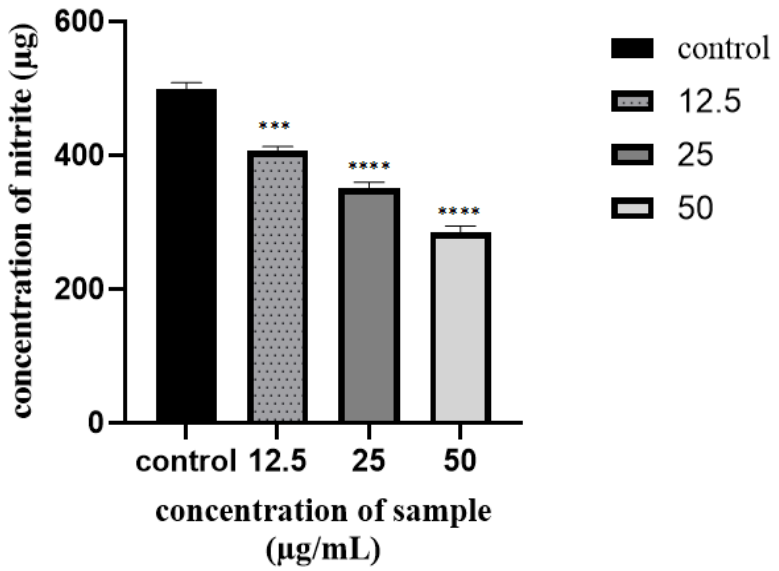

Diagram 5: Graphical representation illustrating the effect of water extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) on cellular nitrite level. Each data represents the Mean ± SE of three replicates.

DISCUSSION

Using sample concentrations of 12.5 µg/mL, 25 µg/mL, and 50 µg/mL, anti-inflammatory tests such as Cyclooxygenase activity, Lipoxygenase activity, Myeloperoxidase activity, Inducible nitric oxide synthase, and Estimation of cellular nitrite levels were analyzed in this study. The proportion of inhibition of cyclooxygenase activity increased as sample

concentration increased in the assay. The percentage of cyclooxygenase activity inhibition was 12.723% at 12.5 µg/mL, 33.841% at 25 µg/mL, and 49.238% at 50 µg/mL and found highly significant.At 12.5 µg/mL of sample concentration, the percentage of inhibition of lipoxygenase (LOX) activity was 11.002%; at 25 µg/mL, it was 37.55%; and at 50 µg/mL, it was 49.494%. The percentage of activity inhibition rises in tandem with the sample concentration. Lipoxygenase (LOX) activity was found to have moderately significant anti-inflammatory activity at sample concentrations of 12.5 µg/mL and highly significant anti-inflammatory activity at sample concentrations of 25 µg/mL and 50 µg/mL.The activity of the enzyme myeloperoxidase (MPO) decreased as sample concentration increased. At 12.5 µg/mL, the enzyme activity was 0.0165 U/ml; at 25 µg/mL, it was 0.0133 U/ml; and at 50 µg/mL, it was 0.0075 U/ml. The enzymatic activity was 0.0199 U/ml when the sample was induced with LPS. After analysis, it was discovered that the enzyme activity was highly significant at all three sample concentrations (12.5 µg/mL, 25 µg/mL, and 50 µg/mL).With respect to inducible nitric oxide synthase, the percentage of inhibition was 12.72% at 12.5 µg/mL, 33.841% at 25 µg/mL, and 49.238% at 50 µg/mL.The activity inhibition % increased along with the sample concentration and was determined to be highly significant at all three concentrations.As sample concentration increased in the estimation of cellular nitrite level activity, nitrite concentration decreased. When nitrite was induced with LPS, its concentration was 499.12 µg. At 12.5 µg/mL, it was 406.89 µg; at 25 µg/mL, it was 350.46 µg; and at 50 µg/mL, it was 284.29 µg. These three different sample concentrations (12.5 µg/mL, 25 µg/mL, and 50 µg/mL) have been found to be highly significant.

CONCLUSION

The anti-inflammatory activity has been analysed using anti-inflammatory assays include Cyclooxygenase activity, Lipoxygenase activity, Myeloperoxidase activity, Inducible nitric oxide synthase and Estimation of cellular nitrite levels and found to be highly significant ($P<0.001$). The study has been statistically analysed by One way ANOVA and Dunnetts multiple comparison test. Indirect ELISA analysis shows the inhibitory effect of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) on Cox-2 antibody activity and thereby showing anti-inflammatory activity.

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