Evaluation of anti-inflammatory activity of *Tinospora Cordifolia* extract through in-vitro and in-vivo methods

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Abstract

Rheumatoid arthritis (RA) is a systematic autoimmune disease that causes joint inflammation, pain and stiffness, leading to loss of function of the joint. However, pharmacological treatments are usually accompanied by undesired adverse side effects, therefore, there has been a relatively increased concern in other forms of treatment. In this research, the possible treatment effects of *Tinospora cordifolia* are examined given that the plant possesses anti-inflammatory and immunomodulatory properties. Thus, *in vivo* and *in vitro* approaches were employed in conducting the research. *In vivo* the study entailed the use of *Tinospora cordifolia* extract on an animal model of RA to determine its therapeutic efficacy in relation to joint swelling, pain and inflammation. Some of the animals received the extract for a pre – determined period and clinical signs of arthritis like paw swelling and pain score were assessed. The changes in the degree of tissue injury and inflammation at the cellular level were also assessed using histopathological examination. *In vitro* studies analyzed the scientific performance of the extract on immune cells and secretion of inflammatory cytokines. PBMCs were prepared and cultured with *Tinospora cordifolia* extract and the levels of TNF-α and IL-1β, IL-6 were estimated. Cell viability and the regulation of cytokine gene expression were measured by enzyme-linked immunosorbent assay (ELISA) and quantitative real-time PCR, respectively. The findings showed that the tested compounds reduced the joint inflammation and pain in the *in vivo* RA model, as well as suppressed the generation of pro-inflammatory cytokines in the *in vitro* experiments. It is further evidence that *Tinospora cordifolia* may have immunomodulatory effects and anti-inflammatory activity and could be used as a complementary or even an alternative to conventional RA medicines. Thus, more studies are required before pinpointing bioactive compounds accountable for the effects depicted herein.

Introduction

Inflammation, which is analyzed from the Latin word "*inflammation"*, which translates to mean "to set on fire" is a biologic process that is often essential but intricate that results from tissue injury or microbial invasion.[1,2] It is defined as the body's response to clear out offending agents and debris, on the offense against disease, and to reconstruct a healthy internal environment. However, inflammation is a quite useful process in the defence of the human organism, with a disproportionate expression of pro-inflammatory and anti-inflammatory factors, it turns into a pathological one.[3] Proliferative inflammation is the rapid, short cycle response to any form of harm like infection, injury or pathogen and its symptoms are redness, heat, swelling and pain. In some cases, the inflammation may be prolonged by the presence of a constant cause or by a malfunction in the regulatory system, which may lead to the appearance of various diseases.[4]

Inflammation is also considered as a root cause of several types of persistent ailments including cardiovascular diseases, diabetes, cancer and autoimmune diseases like RA.[5] RA is an autoimmune, non-familial, inflammatory disease of unknown etiology which causes diffuse inflammation in the synovial membrane of joints and may also involve other organs and tissues.[6,7] It is best described by chronic synovial inflammation, causing joint lesions, pain, deformity, and limitation of movement. In RA, the body's immune system misfires and turns on the synovium, which leads to inflammation, cartilage breakdown, and bone loss.[8] It has a T-cell dependent B-cell mediated immune response through the production of various cytokines that are immunopathogenic and cause inflammation and tissue pathogenesis. Currently, RA affects about 1% of the world's population; it is a leading cause of disability, and the immune system disease reduces quality of life and work output. The treatment of RA has embraced the use of NSAIDs, glucocorticoids together with DMARDs to address more of the symptoms

while attempting to reverse the progression of the disease. However, the treatments mentioned above have their drawbacks, they cause side effects, are not always effective, and can provoke long-term consequences. The growing desire for therapies that are less likely to have side effects, and which can treat the cause of inflammation, has led to a rise in the use of natural products, especially herbs.

Tinospora cordifolia, a plant of the *Menispermaceae* family, is commonly called Giloy or Guduchi and has been conventionally used in medicine due to its therapeutic values. Ginger is known to possess anti-inflammatory, immunomodulator, antioxidant and hepatoprotective activity. The plant *Tinospora cordifolia* has been classified as a Rasayana herb in Ayurvedic medicine since it is used to treat diseases that rejuvenate the human body and enhance energy. It has been used in the treatment of ailments that range from fever, inflammation, infections to digestive ailments. The research carried out in the last decade has put emphasis on the possibility of using it as an anti-inflammatory drug in conditions like arthritis, because of its immunomodulatory and antioxidant effects.

T. cordifolia possesses a rich profile of bioactive constituents including alkaloids, flavonoids, triterpenoids, and phenolic acids, which all are expected to be pharmacological effective.[9] These compounds exhibit characteristic features of being anti-inflammatory and analgesic and thus may be of value in curing some inflammatory diseases such rheumatoid arthritis.[10] The plant also exhibits antioxidant properties, the ability to counter the actions of free radicals that promote inflammation and joint destruction in RA.[11] Moreover, studies have suggested that *Tinospora cordifolia* could have immunomodulating potential of the immunity in RA by controlling the inflammation response of the immune system and avoiding the autoimmunity reactions of the RA type.[12,13]

The objective of the current research in this area will be to evaluate the efficacy of *Tinospora cordifolia* extract on inflammation, oxidative stress and arthritis using in vitro and in vivo models. The current work will therefore examine the ability of the plant to reduce inflammation and joint destruction in RA through the regulation of the following inflammatory mediators. Since RA and similar conditions are on the rise and conventional treatments present certain drawbacks, discovering herbal medicines like *Tinospora cordifolia* can prove beneficial to existing treatment methods that are not only safer, but more efficient as well.

Materials and methods

Drugs and Reagents

Sodium chloride (NaCl), sodium hydroxide (NaOH),ascorbic acid, diphenyl-1 picrylhydrazyl(DPPH),hydrochloricacid(HCl),iodine,dinitrosalicylicacid(DNS),sodiumpotassiumtartarateof analytical grade were used. Normal saline was procured from Medipak Limited®, Pakistan. Carageenan lab grade, Xylene Lab grade, Diagnostic kits (for ALT, AST cholesterol, triglyceride, Urea,Creatinine).

Equipment’s

The double beam spectrophotometer (Shimadzu UV-1601, Japan), digital electronic analytical balance (Galvano Scientific, Pakistan), rotary evaporator (RE-501, Germany), vortex mixer (VM-300), ultrasonic processor (Raheel and Sons), UV-Visible detector (SPD-10AV), centrifuge apparatus (Centurion Scientific, UK), homogenization device (Wise Tis HG-15A), refrigeration unit, digital water bath

apparatus (HH-S41, China), and binocular stereo microscope (YJ-T5C, China) are all integral pieces of laboratory equipment.

Collection of plant Extract

Tinosporacordifolia plants were taken from a residential garden. The plant was characterized by the botanist Prof. Dr.Arif Malik. Identification number:**- IOP(313/IMBBIipo3291/12/22).** The stem was sun-dried, crushed, and ground to a coarse powder. The triturate was macerated for 14 days while being agitated regularly. To set apart the liquid from the solid residue, an aqueous ethanolic extract having ethanol to water (70:30) was filtered. The liquid extract was evaporated using a rotary mixing apparatus at 40°Celsius in decreased pressure for 5–6 hours before being freeze dried, to get a semisolid mass, the extract was maintained in the refrigerator at 2–8°C. For future usage, the plant extract was stored at room temperature (Mishra,et al 2014).

Phytochemical analysis

Phytochemical analysis was performed by dissolving 1g of plant extract in 100ml of solvent to prepare a stock solution for subsequent tests. In the alkaloid test, 2 ml of the filtrate was treated with 2–3 drops of Mayer's reagent, and the assembly of a white, creamy precipitate confirmed alkaloids. For Wagner’s test, 2–3 ml of Wagner's reagent was adjoined to 2 ml of the filtrate, producing a reddish-brown precipitate to confirm alkaloids. In Hager’s test, 1 ml of Hager's reagent was added to 1 ml of extract, and a pale-yellow precipitate confirmed alkaloids. To detect reducing sugars, equal amounts of extract and Benedict's reagent were mixed and boiled, producing a reddish-brown precipitate to indicate carbohydrates. In Millon’s test, 2 ml of filtrate and Millon’s reagent formed a white precipitate, confirming proteins.

In-vitro Activity

The antioxidant ability of the herbal plant extract was assessed utilizing the DPPH inhibition method. A 0.4 mM DPPH solution was synthesized by dissolving 40 mg of DPPH in methanol to a final volume of 100 ml. The control solution consisted of 0.2 ml DPPH solution and 2 ml methanol. The extract’s activity was tested by mixing 1 ml of ethanol, 1 ml of the test solution, and 2 ml of DPPH solution in each test tube. The tubes were covered with aluminium foil, kept in the dark at room temperature (20–25%), and incubated for 30 minutes. Assimilation at 517 nm was determined using a spectrophotometer, and the percentage of DPPH inhibition was calculated.

$(A_0 A1) / (A_{0\,X} 100) = \text{percentage inhibition (percent)}$

Where, $A_0 =$ Absorbance of control

A1 denotes the standard sample absorbance.

HRBC membrane stabilization method

The anti-inflammatory potential of ethanolic *Tinosporacordifolia* stem extract was evaluated using the HRBC membrane stabilization technique. Blood was acquired from a healthy volunteer, mixed with Alsager’s solution, and centrifuged with normal saline. To 1 ml of the HRBC suspension, equivalent volumes of extract at concentrations of 250 mg/kg, 500 mg/kg, and 750 mg/kg were added. The mixtures

were incubated at 37°C for 30 minutes, then centrifuged. The hemoglobin in the supernatant was measured with a spectrophotometer at 560 nm, and the percentage of hemolysis was assessed

% Anti-Haemolysis Activity = Absorbance of control – Absorbance of sample/ Absorbance of control x 100

Protein de-naturation assay (dup: abstract ?)

The modified BSA assay was used to assess the extent of anti-inflammatory potential of *Tinospora cordifolia* extracts. Alcoholic solution of the plant extract at a concentration of 50 µg/mL was prepared and then combined with BSA solutions in Tris-Buffered Saline solution, pH 6.4 at concentrations of 0.25 µg/mL, 0.50 µg/mL and 1.00 µg/mL. The mixtures were incubated at 72°C for 10 minutes, cooled on ice and centrifuged afterwards; the supernatants were then used to measure the protein turbidity at 660 nm. The procedure was carried out in duplicate to determine the percentage inhibition of protein denaturation.

% Anti-Denaturation Activity = Absorbance of control – Absorbance of sample/Absorbance of controlx100

Experimental animals

The Wistar rats two weeks old of either sex were collected and acclimated in the Riphah Institute of Pharmaceutical Sciences' animal house (RIPS). Standard laboratory conditions were supplied to all animals. The room was kept at a constantly set temperature of 25°C, with a humidity level of 55–70 percent. There were 12-hour light and dark cycles (Capdevila et al., 2007). During the trial, the treatment group rats were fed a regular food, whereas rats including normal control group were fed a standard diet. (Sahin et al., 2007).

Anti-Arthritic property

Anti-arthritic activity was the tested using arthritic model rats, Arthritis was induced in rats by injecting 0.1 ml of Complete Freund’s Adjuvant (CFA) to the left hind paw. The paw edema induced by carrageenan was evaluated using digital micrometer gauge on day 0, 3, 7, 18 and 21 after treating with Tinospora cordifolia extract. Paw edema percentage growth was calculated using the formula: This was calculated as; $((T0 - T)/T0) \times 100$, where T0 represents the initial paw thickness and T represents the paw thickness at a certain time. These data were compared with CFA controls, and methotrexate at a dose of 2.5 mg/kg/week was used as the reference anti-inflammatory therapy.

Radiological Assessment

Radiographs were collected with an X-ray device on day 22 after CFA injection (PHILIPS Diagnose X-ray, Amsterdam, Netherlands). Accordingly to the damage level of osteoporosis, joint spaces, inflammation in the soft tissues, erosion of joints and jointankylosis, the severity of the joint deformation was blindly evaluated on a scale of 0–4 as indicated by.Per paw, the highest possible score is 20. The qualified radiologist that were blinded to the therapy groups, evaluated and assessed X-ray pictures individually.

Eddy's hot plate method (Analgesic potential)

The animal was placed on a hot plate for acclimation (3–5 minutes), with a cut-off period of 18 seconds and a maximum heat of 55.1°C to prevent harm. The reaction time, measured in seconds, was recorded when the animal began paw licking or jumping. After oral administration of *Tinospora cordifolia* extract, the test was repeated at 30, 60, 90, 120, and 180 minutes. Each animal was tested three times at 5-minute intervals. Latency time was calculated using the appropriate equation.

(T-To/Tox100)

where Tt, is time therat takes to react at a particular measurement of time and To is the time the rat takes to respond at the initial time 0.

Xylene Induced Ear Odema

In ongoing study, hence the impact of ethanolic *Tinospora cordifolia* extract on xylene induced ear oedema in Wistar rat from 250, 500 and 750 mg/kg doses of the extract were examined after the application of 0.02 ml of xylene. The control group was treated with diclofenac gel, whereas aspirin standard treatment (5 mg/kg) was used. Ear thickness was determined using micrometer screw gauge at 3 hours after induction. Six groups of rats were made: Control, untreated control and treated with different concentrations of the extract or standard pharmacological agents.

Percentage inhibition of ear thickness was assessed using the following formula:

Percentage inhibition of ear thickness = (v_O–$v_{t/}V_C$ ×100)

wherev_t is the ear thickness in average, in the treated groups and v_c is the average ear thickness in millimeters of control group.

In-vivo study

Arthritic rats were separated into six groups, each with six Wistar rats. Treatment began on day 3 after Complete Freund's Adjuvant (CFA) induction and continued for 14 days. Group 1 (normal control) received 5 ml/kg/day NaCl, Group 2 (diseased control) received saline, and Group 3 (standard therapy) received 2.5 mg/kg weekly Methotrexate. Groups 4, 5, and 6 were treated with 250 mg/kg, 500 mg/kg, and 750 mg/kg/day *Tinospora cordifolia* extract, order wise. All treatments were administered orally once a day.

Weight variation

The rats weight were noted at initial, 7th and 14th day of study. Effect of *Tinosporacordifolia*extract on body weight ofthe rats were observed.

Preparation of Tissue Homogenate

To make the homogenate, pancreatic, hepatic and kidney tissues obtained from slaughtered rats, carefully cleaned with ice chilled normal saline before being diffused in 0.1 M phosphate buffer (7.4) 10 percent w/v of tissue.

Protein estimation

Lowery came up with a method of estimating proteins in the kidney, liver and pancreas. To 5 ml of tissue samples diluted with phosphate buffer, 5 mL of copper sulphate containing 1% sodium carbonate and 2 mL of a sodium potassium tartrate were added. Then, the solution was incubated for 10 minutes. After that 0.5 ml Folin-Ciocalteau phenol reagent was added togather. This was followed by mixing the combination for an additional 30 minutes. At 620 nm, the absorbance was assessed.

Following formula has been used to evaluate the protein level.

$Y = 0.00007571x + 0.00004762$

A previously established approach was used to assess CAT activity. A cuvette having 1.95 ml of buffered phosphate solution (pH 7.0, 50 mM) and 1 ml of H_2O_2 had a 1 ml supernatant added to it (H_2O_2 30mM). At a wavelength of 240 nm, the absorbance change was recorded every 5 seconds for 30 seconds. The CAT activity was measured in unit/mg of protein and compared to the standard. At 25°C, one CAT activity unit equaled 1 M of hydrogen peroxide that detoriated into water, per minute.
Catalase activity = δ O.D. / E x Vol. of sample (ml) × mg of protein

δOD = The Change inabsorbance/minute;

E = Co-efficient of extinction (0.071 $mmolcm^{-1}$).

Superoxide dismutase

Free radical scavenging ability of the tissue homogenates was investigated. In the alkaline media auto oxidation of pyrogallol produced these radicals. Each of the 3 mL combinations consisted of 2.8 mL of buffered potassium phosphate (0.1 M, pH 7.4), 0.1 mL of pyrogallol solution (2.6mM in 10mM HCl) and 0.1 mL of tissue homogenate. The absorbance changes for 5 minutes were recorded at 325 nm interval every after 30 secs.Each SOD unit was equivalent to the number of enzyme needed to inhibit pyrogallolautoxidation by 50% under the test conditions

Liver function test

Alanine aminotransferase known as (ALT) and Aspartate aminotransferase denoted as (AST) were analyzed by standard methods (Mansouri et al., 2011).

Renal function test

Urea and creatinine levels were determined by the established standard methods (Mansouri et al., 2011).

Interleukin-6

Standard dilution was prepared by serially transferring solutions through test tubes. For sample preparation, 40 µL of sample was mixed with 10 testing samples, incubated at 37°C, and diluted 30-fold with distilled water. The wells were washed with buffer, followed by the addition of HRP-conjugate reagent and further washing. Chromogen solutions A and B were applied, and after a 15-minute incubation at 37°C, the reaction was stopped by stop solution addition.

$Y = 0.0043x + 0.0466$

TNF-Alpha level

To calculate tumour necrosis factor alpha, standard dilution was prepared by serially transferring 100 µl of a 1350 pg/ml standard solution from tube to tube up to the ninth. For sample preparation, 40 µl of the sample was mixed with 10 testing samples, coated with adhesive, and incubated at 37°C for 30 minutes. The solution was diluted 30-fold, washed, and HRP-conjugate reagent was added. Chromogen solutions A and B were applied and incubated at 37°C for 15 minutes, avoiding light. The reaction was stopped by adding 50 µl of stop solution, causing a color change from blue to yellow.

Cycloxygenase − 2 level

COX-2 was estimated by first preparing a serial dilution of a 1350 pg/ml standard. Sample dilutions (40 µl) were mixed with testing samples, incubated at 37°C for 30 minutes, and then diluted 30-fold with

distilled water. The wells were washed, and HRP-Conjugate reagent was added, followed by incubation and further washing. Chromogen solutions A and B were applied, and after incubation, the reaction was stopped with a stop solution, causing a color difference from blue to yellow. Absorbance was assessed at 450 nm.

$$Y = 0.0003x + 0.1509$$

Carrageenan induced paw edema

Wistar rats of both sexes in the weight range of 120–170 g were obtained from the Riphah International University animal house and were maintained on a standard diet and water ad libitum. For determination of the level of anti-inflammatory activity, carrageenan induced rat paw edema model was employed. Six groups were administered with varying concentrations of the plant extract prepared in sterile water and injected intraperitoneally. After one hour, the animal received 0.1 ml of 1% carrageenan suspension intrapaw into the right hind paw. The paw circumference was measured every one hour for three consecutive hours. On the edema, anti-inflammatory effect was determined using piroxicam (10 mg/kg) as reference drug.

Statistical Analysis

Calculated data were represented in the shape of Mean ± SEM. One and two way ANOVA tests were applied to the collected data, using Graph pad prism (CA, USA, San Diego). The Analyzed results were considered as significantly moderate when ($p \leq 0.01$) while they were considered as significantly high when ($p < 0.001$).

Results

Detection of phytochemical

Phytochemical analysis was used to determine the presence of various components in an ethanolic extract of *Tinospora cordifolia* for qualitative analysis. The presence of alkaloids, saponins, flavonoids, phenols, tannins, and steroids was discovered after the experiment. All other phytochemicals were absent from the plant extract.

Table.1 **Phytochemical analysis of Tinospora cordifolia extract**

Secondary metabolism	Test	Results
Carbohydrates	Fehling’s test	Negative
	Benedict’s test	Negative
Alkaloids	Mayer’s test	Positive
	Wagner’s test	Positive
	Hager’s test	Positive
Phenols	Ferricchloride Test	Positive
	Gelatine test	Negative
Tannins	Lead acetate test	Positive
Flavonoids	Alkaline reagent Test	Positive
Saponins	Foam test	Positive
Gums and mucilage	Gum test	Negative
Steroids	Steroid test	Positive
Glycosides	Bontrager’s test	Negative
	Legals test	Negative
Fixed oil/fats	Spot test	Positive
Proteins and amino acids	Millon’s test	Negative
	Biuret test	Negative
	Ninhydrin test	Negative

DPPH %age inhibition

Antioxidant activity was measured using various doses of plant extract and ascorbic acid, as well as DPPH, to assess the antioxidant capabilities of Tinospora cordifolia ethanolic extract. 1, 0.5, 0.25mg/ml, 0.125mg/ml, 0.0625mg/ml, 0.03125mg/ml, and 0.0156mg/ml were the concentrations employed. Each concentration's absorbance was measured after 30 minutes. Percentage inhibition calculations were made. After using a one-way ANOVA test, the percentage inhibition of ascorbic acid concentrations was substantially higher than all plant extract concentrations antioxidant properties by DPPH method using Tinosporacordifoliaextract and ascorbic acid.

Table.2 Percent DPPH inhibition exhibited by ethanolic extract of plant

Concentrations (mg/ml)	%age Inhibition of DPPH	
	Tinospora cordifolia extract	Ascorbic acid
1	71.0 ± 3.07**	83.4 ± 1.25
0.5	57.8 ± 3.11*	70.3 ± 2.37
0.25	50.2 ± 1.94	67.8 ± 1.26
0.125	43.1 ± 1.27**	54.8 ± 1.22
0.0625	41.0 ± 2.27***	49.9 ± 1.27
0.03125	34.7 ± 1.17**	45.4 ± 1.09
0.0156	28.1 ± 1.22**	37.1 ± 1.15

*The Data were shown as mean ± standard deviation (n = 6), further analyzed by one way ANOVA,then application ofTucky's test. ***(p < 0.001) **(p < 0.01),*(p < 0.05), the values observed were quite significant.*

HRBC membrane stabilization method

The ability to reduce inflammation of various stem extracts of *Tinospora cordifolia* was assessed by the in vitro HRBC membrane stabilization technique. The haemoglobin content in the treated centrifuged solution's supernatant, was estimated by using measuring its absorbance at 560nm. The percentage of haemolysis was calculated by using the following formula:

% Anti-Haemolysis Activity = Absorbance of control – Absorbance of sample/ Absorbance of control x 100

Table 3
Effect of CEE and ASA on heat-induced hemolysis of the RBC membrane

Drug Conc. (µg/ ml)	% Stabilization of Tinospora cordifolia	% Stabilization of Diclofenac sodium
200	73.2 ± 1.2	78.5 ± 1.2
400	78.5 ± 2.1	83.2 ± 1.4
800	84.4 ± 1.4	86.8 ± 1.31*
1200	87.2 ± 1.4	94.3 ± 1.21
1600	89.7 ± 1.22	96.4 ± 1.23
2000	93.5 ± 1.3	97.61 ± 1.6**

*The data were shown as mean ± standard deviation (n = 6), that was analyzed using the one way ANOVA. ***(p < 0.001) **(p < 0.01),*(p < 0.05), the values observed, were found to be quite significant.*

Protein de-naturation assay

The anti-inflammatory potential was evaluatedusing an air blank as a reference, the turbidity of the solutions (amount of protein precipitation) was measured at 660 nm in the Spectrophotometer to test the anti-inflammatory properties of crude plant extracts. Aspirin was employed as a positive control. The

studies were carried out twice, and mean absorbance values were noted. The following calculation was used to calculate the percentage inhibition of precipitation (protein denaturation) incomparison to the negative control:

% Anti-denaturation Activity = Absorbance of control – Absorbance of sample/Absorbance of controlx100

Table 4
Protein de-naturation assay

Drug Conc. (µg/ ml)	% inhibition of Tinospora cordifolia	% inhibition of Diclofenac sodium
200	74.4 ± 1.2	88.5 ± 1.1
400	79.4 ± 2.2	87.2 ± 1.2
800	86.3 ± 1.2	94.4 ± 1.21*
1200	87.3 ± 1.3	96.1 ± 1.1
1600	91.2 ± 1.22	97.1 ± 1.2*
2000	92.3 ± 1.4	98.41 ± 1.2**

*Table 4. The Data given is presented as mean ± standard deviation (n = 6), analyzed by one-way ANOVA. ***(p < 0.001) **(p < 0.01),*(p < 0.05), the values observed, were found to be quite significant.*

Effect of weight

The Animals lost weight in the preliminary stages of the experiment. The weight difference was almost 30 percent. When the rats were treated with standard therapy as well as with plant extract, their weight decreased significantly (P0.01) and the body weights were 251 ± 4.5* ,268 ± 2.1**,257 ± 9.8) to (247 ± 1.5*,249 ± 7.0***,236 ± 3.2) in 2nd week at 7th and 14th day respectively. While the Plant extract demonstrated a substantial reduction. (P0.001) at 500mg/kg concentration as shown in the weight.

Table 5
Effect of *Tinosporacordifolia*extract on weight of arthritic rats

Weight (gm)	Treatment	Basal	Zero day	7th day	14th day	21st day
	Normal Control	284 ± 4.1	285 ± 5.1	289 ± 2.3	270 ± 4.6**	267 ± 6.1
	Diseased control	290 ± 1.5	286 ± 9.1	275 ± 4.1	267 ± 6.3	254 ± 5.6*
	Standard therapy	274 ± 6.4	258 ± 3.3	258 ± 6.1**	242 ± 11.7**	210 ± 6.4
	Plant extract 250mg/kg	282 ± 1.9	271 ± 7.2	251 ± 4.5*	247 ± 1.5*	234 ± 5.3
	Plant extract 500mg/kg	296 ± 7.3*	276 ± 4.5*	268 ± 2.1**	249 ± 7.0***	238 ± 6.3
	Plant extract 750mg/kg	292 ± 1.8	271 ± 6.2	257 ± 9.8	236 ± 3.2	229 ± 7.2**

*The data that were observed were articulated as the mean ± standard deviation (n = 6), subsequently assessed utilizing one-way ANOVA, followed by the application of Tukey's test. ***($p < 0.001$) **($p < 0.01$),*($p < 0.05$) upon the comparative analysis of both the diseased and control cohorts, the values that were observed were significantly pronounced.*

SOD Levels

There was decreased SOD activity in all the test organs in arthritic rats suggesting that the antioxidant defence was compromised. *Tinospora cordifolia* restored antioxidant enzyme levels in the pancreas, an organ commonly affected by arthritis because of autoimmunity. At 250mg/kg (8.9 ± 0.5), 500mg/kg (9.3 ± 0.5), and 750mg/kg (8.1 ± 0.5) concentrations the extract demonstrated a dose dependent trend. However, at higher doses (500mg/kg and 750mg/kg), the enzyme levels declined as reported in the previous trial. In the kernel area, the extract raised antioxidant enzymes of the kidney at 250mg/kg (9.8 ± 0.5), the 500mg/kg (10.1 ± 0.5) and 750mg/kg (11.6 ± 1.0) as compared to the control group (8.13 ± 0.5).

Table 6
Effect of Tinosporacordifoliaextract on SOD (µg/mg of protein) levels in varying organs of arthritic rats.

Groups	Liver	Kidney	Pancreas
Normal control group	8.1 ± 0.5**	14.2 ± 0.5***	13 ± 0.01***
Diseased Group	5.8 ± 0.5	7.81 ± 0.5	8.13 ± 0.5
Standard Group	7.1 ± 0.5*	12.1 ± 0.5***	12.3 ± 0.5***
250 Plant Extract	6.31 ± 0.5	9.8 ± 0.5***	8.91 ± 0.5
500 Plant Extract	6.64 ± 0.5	10.1 ± 0.5***	9.25 ± 0.5*
750 Plant Extract	7.03 ± 0.5*	11.6 ± 1.0***	8.13 ± 0.5**

The compiled data were represented as mean ± standard deviation (n = 6) and analyzed through two-way ANOVA followed by Tukey's test. ***($p < 0.001$) **($p < 0.01$),*($p < 0.05$) Upon analysis, the diseased and plant extract groups demonstrated statistically noteworthy values.

CAT Levels

In all the arthritic rats, the CAT enzyme levels were determined, and it has been found that cat levels rise. In the hepatic, pancreatic and nephritic tissues, CAT activity was significantly higher ($p < 0.001$) in the normal control group (70 ± 2.5, 55 ± 0.5) and (30 ± 0.5) respectively; whereas in all other groups, the CAT activity was reduced. In respect with the conventional treatment group, the cat activity of treat cause was found increased significantly in the liver, kidney and pancreas (65 ± 1.0, $p < 0.0$), (50 ± 1.0, $p < 0.05$) and (26 ± 1.5, $p < 0.001$) respectively. Kidney and pancreas were normal in the 250mg/kg plant extract but liver was reduced (58 ± 1.3, $p < 0.001$) as compared to normal group. The arthritic group treated with 500mg/kg of plant extract showed a significant lowering of CAT activity in the liver (61 ± 0.4, $p < 0.001$) and pancreas (23 ± 0.5, $p < 0.05$) but no effect was observed in kidney. The liver, pancreas and kidney had reduced concentrations of 750mg/kg (63 ± 0.5, $p < 0.01$), (25 ± 1.0, $p < 0.01$), and (50 ± 0.5, p0.05) respectively.

Table 7
CAT Levels

Groups	Liver	Kidney	Pancreas
Normal control	70 ± 2.5***	55 ± 0.5**	30 ± 0.5***
Diseased control	59 ± 2.6	39 ± 0.5	19 ± 0.5
Standard therapy	65 ± 1.0**	50 ± 1.0*	26 ± 1.5***
250 Plant Extract	58 ± 1.5***	42 ± 0.5	20 ± 0.5
500 Plant Extract	61 ± 0.5***	47 ± 0.5	23 ± 0.5*
750 Plant Extract	63 ± 0.5**	50 ± 0.5*	25 ± 1.0**

*The calculated results were displayed as mean + SD ($n = 6$) and assessed using one-way ANOVA and Tucky's test. Results comparing the sick and control groups with ***($p < 0.001$) **($p < 0.01$),*($p < 0.05$) revealed extremely significant findings.*

Renal Function Test

In comparison to the rats in the control group, the sick group's urea levels rose. There was a significant reduction in urea concentration in both the usual treatment group and the groups treated with plant extract at various concentrations ($p < 0.001$). When arthritic rats were compared to control rats, their creatinine levels increased as well. Except for the group treated with 250mg/kg plant extract, which exhibited a significance of $p < 0.01$, the conventional therapy group and groups treated with plant extract at different concentrations showed a considerable drop in urea levels ($p < 0.001$).

Table 7
Effect of Tinosporacordifoliaextract on renal enzymes of arthritic rats

Treatment Groups	Urea(mg/dl)	creatinine(mg/dl)
Normal control	31 ± 3.4***	0.35 ± 0.06***
Diseased control	61 ± 2.3	1.05 ± 0.10
Standard therapy	38 ± 2.6***	0.38 ± 0.07***
250 Plant Extract	95 ± 4.4***	0.81 ± 0.02**
500 Plant Extract	118 ± 2.5***	0.72 ± 0.07***
750 Plant Extract	127 ± 3.4***	0.67 ± 0.03***

*The analyzed data were presented as mean ± standard deviation ($n = 6$) and evaluated by one way ANOVA followed by Tucky's test. ***($p < 0.001$) **($p < 0.01$),*($p < 0.05$) compared diseased and control groups, showed highly significant results.*

Liver function test

ALT level in blood shows whether the cell has been injured by the liver or the degree of cellular dysfunction. Thus, normal medication with the Tinospora cordifolia extract at all doses showed a decrease in the ALT level in all the rats ($p = 0.05 < 0.001$). For the AST levels, the mean was notably high compared to that of the control group. The usual treatment group also showed a decrease of AST levels of 0.5 ± 23 ($p < 0.001$). On the other hand, the AST levels of plant groups appeared to decline progressively. At 250 mg/kg no alteration observed, however, at 500 and 750 mg/kg significant decrease in AST level 161 ± 8 as presented in the table ($p < 0.01$), ($p < 0.001$) and ($p < 0,01$) respectively. Regarding bilirubin, the arthritic rat groups revealed a higher pattern of bilirubin level than the normal control group. Compared to the standard treatment and plant extract at 500 and 750mg/kg, the bilirubin level was also significantly lower ($p < 0.001$). Likewise, in the arthritic rat model the Alkaline phosphatase level were substantially raised than the control group rats, all doses, all treatment and plant extract treated groups showed a marked decrease in Alkaline phosphatase level ($P < 0.001$).

Table 8
Effect of *Tinospora cordifolia* extract on liver enzymes of arthritic rats

Treatment Groups	ALT (U/L)	AST (U/L)	Bilirubin (mg/dl)	Alkaline phosphatase (U/L)
Normal control	45 ± 3***	130 ± 8***	0.41 ± 0.01***	83 ± 22***
Diseased control	64 ± 4	256 ± 5	0.73 ± 0.02	76 ± 5
Standard therapy	63 ± 14***	205 ± 7***	0.56 ± 0.04***	11 ± 6***
250mg/kg Plant Extract	72 ± 12***	197 ± 8	1.03 ± 0.03*	209 ± 4***
500mg/kg Plant Extract	50 ± 13***	181 ± 2**	0.99 ± 0.01***	211 ± 4***
750mg/kg Plant Extract	25 ± 17***	132 ± 2***	0.98 ± 0.02***	51 ± 4***

The gathered data were expressed as mean ± standard deviation ($n = 6$) and further assessed through one way ANOVA, with Tucky's test. ***($p < 0.001$) **($p < 0.01$),*($p < 0.05$)and the values observed were markedly significant among diseased and control groups.

Anti-arthritic property

The anti-arthritic activity was measured after the induction of Complete Freud's Adjuvant 0.1 ml induction in the paws of rats. The ethanolic extract showed marked decrease in paw size by increasing the dose from 250mg/kg to a maximum at 750 mg/kg.

Table.9 Anti arthritic activity of *Tinospora cordifolia of rats*

Treatment Groups	Paw size mm 0 days	Paw size mm 3 days	Paw size mm 7 days	Paw Size (mm) 14 days	Paw size (mm) 21 days
Normal control	2.6 ± 0.1	2.8 ± 0.2	2.7 ± 0.3	2.67 ± 0.2	2.81 ± 0.4
Diseased control	6.9 ± 0.13	6.8 ± 0.13	6.7 ± 0.2	6.5 ± 0.5	6.3 ± 0.2
Standard therapy	6.67 ± 0.12	6.2 ± 0.23	6.1 ± 0.21	5.6 ± 0.20	4.3 ± 0.14**
250 Plant Extract	5.12 ± 0.11**	5.64 ± 0.14**	4.88 ± 0.12**	4.7 ± 0.1**	4.35 ± 0.3**
500 Plant Extract	6.8 ± 0.12**	6.19 ± 0.11**	5.21 ± 0.12**	4.8 ± 0.22**	4.3 ± 0.23***
750 Plant Extract	6.04 ± 0.2**	5.6 ± 0.41***	5.02 ± 0.13***	4.7 ± 0.11***	4.2 ± 0.12***

*The Statistical data were denoted as mean ± standard deviation (n = 6) and evaluated through one way ANOVA followed with Tucky's test. ***p < 0.001 **p < 0.01,*p < 0.05 compared data depicts significant values.*

Anti-inflammatory markers

IL-6 levels

Interleukin-6, a pleiotropic cytokine called IL-6 is produced by neutrophils and monocytes when their receptors are activated, it has a distinct pro-inflammatory effect while regulating adaptive and innate immunity. The synthesis of acute-phase proteins is mediated by IL-6 (C- Reactive proteins) Hepatocytes produce CRP.

Table 10
Interleukin-6 levels of rats treated with *Tinosporacordifolia*ethanolic extract.

Treatment Groups	IL-6 levels
Normal control	2.6 ± 0.25
Diseased control	6.9 ± 0.12 **
Standard therapy	6.67 ± 0.18*
250 Plant Extract	5.12 ± 0.30*
500 Plant Extract	6.8 ± 0.15*
750 Plant Extract	6.04 ± 0.25*

The statistical values of anti-inflammatory markers represented as mean ± standard deviation (n = 6) and assessed through one way ANOVA, Tucky's test was then applied afterwards. ***p < 0.001 **p < 0.01,*p < 0.05 a significant difference was noted, on comparison of both diseased and plant dose level groups.

Estimation of TNF alpha

The ethanolic extract of *Tinospora cordifolia* showed a significant reduction in TNF alpha levels with increasing dose levels. 750 mg/kg dose showed a marked decrease in the TNF alpha levels similar to the normal group.

Table 11
TNF levels after treatment with *Tinopsora codifolia* extract

Treatment Groups	Tumor Necrosis factor-6
Normal control	67 ± 1.1
Diseased control	123.67 ± 2.9 ***
Standard therapy	80.33 ± 1.25 **
250 Plant Extract	93.67 ± 2.95***
500 Plant Extract	97.1± 1.25***
750 Plant Extract	90.33± 2.9***

TNF-alpha values were presented as mean ± standard deviation (n = 6) and evaluated by one way ANOVA test and followed by the Tucky's test. ***p < 0.001 **p < 0.01,*p < 0.05 resultant values were remarkably among compared groups.

Estimation of COX-2

Cyclooxygenases are pro inflammatory mediators which are inhibited the*Tinospora cordifolia* ethanolic extract. The data presented in graph reveals highly significant decreases in the COX-2 levels in all treated rat groups which expressed that test substance *Tinospora cordifolia* ethanolic extract is effective to decrease the expression of cyclooxygenases in treated rats at all doses levels, similar to the animals treated with standard drug Methotrexate at dose level of 2.5mg/kg/week .

Table 12
COX levels after *Tinosporacordifolia* extract treatment

Treatment Groups	Cycloxygenase-2
Normal control	100.33 ± 2.39
Diseased control	167.25 ± 3.25 ***
Standard therapy	107.92 ± 1.27*
250 Plant Extract	127.25 ± 1.97**
500 Plant Extract	123.67 ± 2.25
750 Plant Extract	123.43 ± 2.25

*The cox levels were stated as mean ± standard deviation (n = 6) analyzed through ANOVA test, followed by the Tucky's test. ***($p < 0.001$) **($p < 0.01$),*($p < 0.05$) notable values were observed as compared to treatment groups with the disease control.*

Eddy's Hot Plate

The extract's analgesic efficacy was further assessed using the hot plate technique. Within the restrainer, the rats were placed on a heated plate that was kept at 55°C. The reaction time (in seconds) or latency period was calculated as the time it took for the rats to lick their paws or leap in response to the thermal discomfort. The reaction time was measured before (0 min), 15, 30, 45, and 60 minutes after the treatments were administered. To avoid harm to the paw tissues, the maximum response time was set at 45 seconds. Maximum analgesia is defined as a reading that exceeds 45 seconds. The following formula was used to get the maximum possible analgesia. The Eddy's hot plate method was applied to check reaction time as observed, starting from 30 sec and gaining the peak effect at 90min. Results were almost two times increase in paw licking time as compared to normal control group. The highest significance was measured at 750 mg/kg dose levels.

Table 13
Reaction time in seconds with different drug dosages in hot plate model of pain in mice Reaction time in seconds

Drugs and doses (mg/kg)	Reaction time At time 0 minutes	Reaction time After 30 minutes	Reaction time After 60 minutes	Reaction time After 90 minutes
Normal control	16s ± 2s	15s ± 1	14s ± 2s	16s ± 3s
Tinosporacordifolia 250 mg/dl	15s ± 1s	19s ± 4s	21s ± 2s	23s ± 3s
Tinosporacordifolia 500 mg/dl	14s ± 3s	19s ± 3s	22s ± 1s	24s ± 2s***
Tinosporacordifolia 750 mg/dl	15s ± 2s**	19s ± 3s**	24s ± 2s**	25s ± 4s**

*The Eddy's hot plate method values were showed as mean ± standard deviation (n = 3) which were evaluated through one way ANOVA and Tucky's test. ***($p < 0.001$) **($p < 0.01$),*($p < 0.05$) significance*

among values were observed.

Carrageenan induced paw edema

The Carrageenan was used as an inflammatory inducer in wistar rats, weighing (120–170 g)along with administration of tinosporacordifolia stem extract of 250,500 and 750 mg/kg.The readings were measured after interval of 1 hour up-to 3 hours,which was calculated by the help of verniercalipers results of which are as follows:

Table 14
Carrageenan induced Paw edema

Extracts	Doses (mg/kg, ip)	Paw edema mean (mm)1 Hr	Paw edema (mm) 2Hr	Paw edema (mm)3 Hr
Control (NS 0.9%)	0.3 ml	3.8 ± 0.05	3.79 ± 0.03	3.788 ± 0.04
Disease control	No drug	6.30 ± 0.21	6. 08 ± 0.34	8.99 ± 0.76
Standard control (piroxicam)	10mg/kg	5.65 ± 0.15	7.47 ± 0.67	7.7 ± 0.34
Ethanolic extract (Tinosporacordifolia)	250mg/kg	6.15 ± 0.88	6.42 ± 0.78	7.63 ± 0.54*
	500mg/kg	5.83 ± 0.90	6.67 ± 0.79	9.6 ± 0.65**
	750mg/kg	6.64 ± 0.78	6.115 ± 0.90	6.5 ± 0.67*

*The Carrageenan induced inflammation was stated as mean ± standard deviation (n = 3) and was evaluated through one way ANOVA along with Tucky's test. ***($p < 0.001$) **($p < 0.01$),*($p < 0.05$)the values were notable among the groups.*

Xylene Induced Ear Odema:

Xylene-induced edema on right ears of wistar rats Effects of ethanolic fraction of *Tinosporacordifolia*on xylene-induced edema on the right ears of wistar rats were investigated following the procedure

Table 15
Xylene induced Ear edema

Extracts	Doses (mg/kg, ip)	Ear edema mean (mm) 1 Hr	Ear edema (mm) 2Hr	Ear edema (mm) 3 Hr	Ear edema (mm) 4Hr
Control (NS 0.9%)	0.3 ml	0.21 ± 0.05	0.23 ± 0.04	0.22 ± 0.03	0.212 ± 0.02
Disease control	No drug	0.88 ± 0.2	0.915 ± 0.3	0.90 ± 0.5	0.902 ± 0.1
Standard control (aspirin)	5mg/kg	0.73 ± 0.15	0.705 ± 0.17	0.675 ± 0.4	0.601 ± 0.13
Ethanolic extract (Tinosporacordifolia)	250mg/kg	0.605 ± 0.6	0.634 ± 0.5	0.61 ± 0.4	0.58 ± 0.2*
	500mg/kg	0.55 ± 0.4*	0.614 ± 0.6*	0.625 ± 0.3	0.57 ± 0.12**
	750mg/kg	0.61 ± 0.3**	0.615 ± 0.03	0.59 ± 0.6*	0.54 ± 0.118**

*The Xylene induced ear oedema results were expressed as mean ± standard deviation (n = 3) and analyzed by one way ANOVA. Tucky's test showed highly significant values. ***($p < 0.001$) **($p < 0.01$),* ($p < 0.05$)on comparison with diseased and control group.*

X-ray of Arthritic rats

The animal paws were preserved in formalin and then x ray diffraction pattern was studied after one day of animal was necropcised. The ethanolic extract of *Tinosporacordifolia* showed that the rat paws showed a decreased inflammation by increasing dose of the drug extract (*Tinosporacordifolia).*The dose response was maximum at 750 mg/kg drug concentration, which was close to the standard drug therapy (Methotrexate 2.5mg/kg/week).

Discussion

In the present study the various *invitro* and *invivo* procedures were evaluated to check the potential anti-inflammatory effect of *Tinosporacordifolia* stem extract. The chemical characterization of *Tinosporacordifolia* extract was analyzed by phytochemical analysis detection methods and it was observed that it contains alkaloids, phenols,tannins, flavonoids, saponins, steroidal compounds and fixed oils components but does not contains carbohydrates, gums and mucilages, glycosides and proteins and amino acid contents. However, in another research, phytochemical analysis of this plant, contained alkaloids, steroidal comounds while with the absence of carbohydrates, pheols, tannins, flavonoid, saponins, gums and mucilages, glycosides and protein and amino acids components.[14]

Diphenyl picrylhydrazyl (DPPH) is a free type of radical and scavenging compound.[15] In the recent study the percentage DPPH inhibition potential was checked by a standard ascorbic acid by dilution method and the inhibitory potential was checked to be maximum at 1 mg/ ml ethanolic extract concentration.[16] The experiment was performed by making serial dilutions starting from 1mg/ml uptil seventh dilution i.e. 0.0156mg/ml and having a standard ascorbic acid. The *Tinosporacordifolia* stem extract showed a maximum value of percentage DPPH inhibition at 1mg/ml i.e. 71% as compared to ascorbic acid i.e. 83.8%. The findings demonstrated that the percentage scavenging potential of *Tinosporacordifolia* was not greater than that of ascorbic acid. The weight was measured weekly in *Tinosporacordifolia* extracts showed a significant loss of weight, decreasing food intake and decreasing body weight in a study doneby the effect was due to the ability of plant extract to reduce fat and decrease appetite in the rats. Super oxide dismutase plays acts as contributing factor against reactive species such as superoxide negatively charged species(anion) and decreases its concentration by converting it into unreactive H_2O_2 and molecular O_2, thus provides protection against pancreatic cellular breakdown. In our study, it was discovered,that plant extract showed a dose dependent action against these free radicals and increased the concentration of pancreatic SOD at 500 mg/kg and 750mg/kg dose level. At 500 and 750 mg/kg dose level the SOD level rose to maximum and respectively as compare to diseased group animals. This shows the decrease of oxidative stress with an increasing dose level. In liver and kidney, plant extract showed significant increase of SOD levels. It is also in our findings that the *Tinospora cordifolia* exhibits a good amount of Vitamin C (41.36 mg/g of extract) and glutathione (6.86 mg/g of extract). Both of these are agents are known to be agents involved in direct scavenging of a wide variety of free radicals.[17] The mechanism of catalase activity was through conversion of H_2O_2 into H_2O and molecular O_2. There exist a dose dependent effect in the cells of pancreas for catalase activity. Plant extract with dose level of 500 mg/kg and 750 mg/kg did produce a significantincrease in CAT levels as compared to diseased groups in all organs[9]. Liver enzymes were decreased by increasing dose levels in all arthritic rats as compare to

normal control group. The reason of these high levels could be the possible leakage from cellular cytosol towards blood stream. Hepatotoxicity leads towards perforation of cell membranes which leads towards leakage. Plant extract did show a protective effect of liver tissues by reversing organ damage, AST and ALT levels were significantly decreased ($p < 0.001$) by treatment of maximum dose of plant extract. Bilirubin and alkaline phosphatase were observed to be significantly decreased ($p < 0.001$) at 500 and 750 mg/kg dose level of plant extract as compare to diseased control group.[18] HRBC membrane stabilization is a test to check the lysosomal integrity as the RBC membrane is quite fragile and is comparable to lysosomal membranes which depicts the protection against denaturation of cellular proteins by preventing the loss of membrane protein breakdown in the lysosomes. HRBC was checked at 560 nm starting from doses 200 to 2000 microgram/ml showing the maximum value at 200 microgram/ml. Hence understood that lysosomal membranes are as fragile as RBC membranes so protection of RBC membrane is equivalent to lysosomal membrane protection and prevention of free radical spilling in the cell as lysis of lysosomes releases H_20_2 and free radicals which causes cellular breakdown.

Protein denaturation assay involves the production of auto antigens which are released by the denaturation of proteins which in turn causes arthritic and inflammatory diseases.[19] The possible mechanism of denaturation of proteins is an alteration in secondary structure and tertiary structural level of proteins due to stress, heat, solvents, strong acids and alkalies.The NSAIDS (Non-steroidal anti-inflammatory medications) used for the cure of rheumatoid arthritis to prevent the breakdown of proteins.[20] In the current study *Tinosporacordifolia* was found to prevent the egg albumin breakdown in a dose dependent manner but the results were lower as compared to the standard drug i.e. Aspirin. Renal function test values were quite improved as comparable to diseased control group showing maximum improvement in 750mg/kg plant extract.The urea and creatinine levels are markers of kidney function stating the removal of these toxins from the body. *Tinosporacordifolia* improves the renal function by protecting the cellular integrity and decreasing levels of free radicals in the cells thus facilitating removal of toxins from the blood. Anti-arthritic property was measured by the induction of complete Freud's adjuvant in the rat paws. The ethanolic extract of *Tinosporacordifolia* demonstrated a dose-dependent decline,in the inflammation of rat paws, decrease in inflammatory activity was due to the bone remodeling activity of the drug and its activity that increases bone density in inflamed tissues by CFA. Interleukin 6 is an inflammatory mediator produced during the inflammatory cascade, it is a pro inflammatory chemical that is responsible for initiation of inflammatory cascade. The *Tinosporacordifolia* shows a marked decrease in interleukin 6 levels due to its immune-modulatory activity by the presence of flavonoids and phenols which are responsible for the reduction of free radicals. It also contains vitamin c and glutathione which are potent anti-inflammatory compounds. TNF-alpha is a cytokine which is a pro-inflammatory chemical which binds to different types of receptors found in all cells except RBC's showing a negative effect on several inflammatory conditions.[21] The *Tinosporacordifolia* stem extract showed a significant effect that was lower as compared to diseased group. It inhibits cellular regulation of Tnf-alpha which decreases the values of Tnf-alpha and other chemical mediators of inflammation.[22] Eddy's hotplate measures the response of animal to external heat .the response time is measured in seconds and the animal is placed on hotplate designed to transmit heat to paws of animals and has a transparent upper part by which we can observe the animal. The tinosporacordifolia stem extract showed a marked decrease in reaction time in response to heat having maximum efficacy at 750mg/kg.

The ethanolic concentrate of *Tinospora cordifolia* was effective in the eddy's hotplate model, demonstrating its likely impact through peripheral and central mechanisms. The action of *Tinospora cordifolia* can be credited to a few plant parts. Protoperberine alkaloids, terpenoids, glycosides and

polysaccharides. furthermore it might be accredited a strong pain killer.[23] Carrageenan is a (polysaccharide) we get from consumable red sea weeds. Carrageenan induced inflammation was checked by injecting carragenan in the paws of wistar rats and their paw sizes were measured up-till 3hrs. The drug *Tinosporacordifolia* showed a remarkable decrease in paw size after 3hrs of inflammation showing a dose dependent increase in response in the anti-inflammatory activity.The irritation incited by phlogistic specialist carragenan is dual in nature. The principal stage is bradykinin and cyclooxygenase release while second stage is connected with neutrophil invasion, as well with respect to the going on of the creation of arachidonic acid metabolites. Prostaglandins and nitric oxide blend is associated with irritation, and the nitric oxide synthase (NOS) furthermore, of cyclooxygenase (COX-2) are theresponsible agents for the creation of an extraordinary measure of these chemicals. The anti inflammtory activity of *Tinosporacordifolia* is due to the presence of flavinoids alkaloids and phenols which decrease the production of inflammatory medicators like IL-6, Cox, and bradykinins. Xylene is a chemical related to the drug xylocaine it is a lab chemical used for the desensitizing of skin and related areas topically. Our study was designed to apply xylene over the ears of rats and measured their thickness before and after the xylene application. The extract of Tinospora showed a considerable decrease in ear thickness size with increasing the dose of the drug.it is due to its phytochemical constituents i.e flavinoids, phytochemicals, having a rapid effect on the inflammatory process. X-ray is a standard technique to evaluate the bone status and its inflammation in the rats, we performed x ray diffraction after necropsizing the animals. The*Tinosporacordifolia*ethanolic stem extract was very effective in the remedial treatment of inflammation as shown in the previous experiments, it has a marked anti-inflammatory activity starting from 250 mg uptill a maximum at 750 mg/kg. the stem extract also increased bone density and a significant decrease in inflammation in the joints after complete Freud’s agent administration (CFA). Bone is a metabolically dynamic tissue that goes through consistent rebuilding over the course of life by cells known as osteoblasts and osteoclasts.[24] This displaying empowers the skeletal framework to occasionally develop and to fix its primary harms. TC extract showed estrogen like effects in the bone tissue but not in the reproductive organs. This study showed that *Tinosporacordifolia stem extract* has a potential for being used as antiosteoporotic drug. Chemical evaluation of *Tinosporacordifolia* extract showed the presence of various phytochemicals. Anti-arthritic activity of has been demonstrated through a number of studies.

Conclusion

The study showed that *Tinospora cordifolia* stem extract exhibited anti-arthritic potential in CFA and carrageenan-induced rats. Phytochemical screening revealed alkaloids, phenols, tannins, flavonoids, saponins, and steroids. The extract reduced pro-inflammatory cytokines, inhibited DPPH free radicals, and demonstrated strong antioxidant potential. It stabilized HRBC membranes, prevented protein denaturation, and reduced paw swelling and body weight loss. Biochemical, oxidative stress, and haematological parameters improved, with near-normalized histopathology of rat hind paws. Increased doses (250 mg/kg to 750 mg/kg) reduced inflammation, improved heat response, and showed reduced inflammation in X-ray studies.

Declarations

This study adhered to all relevant animal ethics guidelines and was approved by the *Institutional Animal Care and Use Committee (IACUC) of Riphah Institute of Pharmaceutical Sciences at Riphah International University Lahore. All procedures were designed to minimize animal pain and distress, utilizing the 3Rs principle of replacement, reduction, and refinement whenever possible. Animals were housed and cared

for in accordance with established protocols, and euthanasia was performed humanely according to accepted veterinary practices.

Conflict of interest

The authors declare no competing financial interest

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Figures

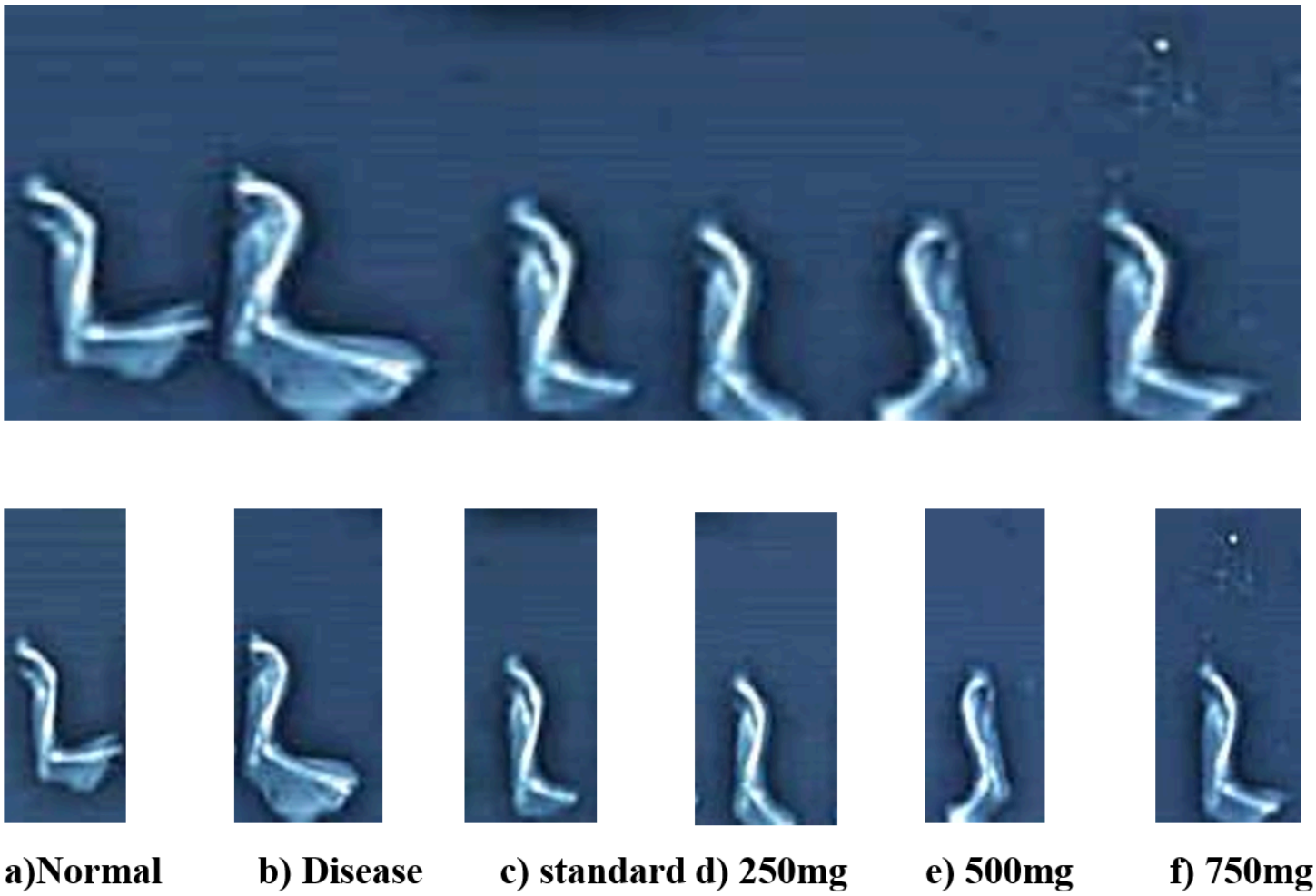

Figure 1

Paw x ray showing swelling of paw

(a) The x ray shows a normal ecotexture of paw having no inflammation (b) The diseased paw showing an increased inflammation of paw (c) The standarad drug (Methotrexate) treated showing decreased inflammation (d) Paw treated with 250 mg/kg dose showing a decreased inflammation (e) Paw treated with 500 mg/kg dose showing decreased inflammation as compared to 250mg/kg (f) Paw treated with 750 mg/kg dose showing very low inflammation closer to the standard drug treatment.