International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-2, 2016 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Preliminary Screening of *Tinospora cordifolia* Extracts and Guduchi Satva for Anti-Rheumatoid Activity

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Manuscript No: IJPRS/V5/I2/00062, Received On: 16/04/2016, Accepted On: 22/04/2016

ABSTRACT

Extracts of *Tinospora cordifolia viz.* aqueous (TCA), hydroalcoholic (TCH), dichloromethane (TCD) extracts and guduchi satva (TCP) (starch), were studied for anti-rheumatoid activity in complete freund's adjuvant (CFA) induced rheumatoid arthritis (RA). Female albino *Wistar* rats (body weight: 170 to 200 g) were divided in 11 groups each containing 3 animals. RA was induced by injecting CFA in sub plantar region of left paw in all animals (day 0) except Group I (normal control). Group II (disease group) received only CFA. Group III (standard group) received methotrexate (0.25 mg/kg). Group IV to XI received low and high doses various extracts *viz.*, TCA1 (50 mg/kg), TCA2 (500 mg/kg), TCH1 (10 mg/kg), TCH2 (100 mg/kg), TCD1 (10 mg/kg), TCD2 (100 mg/kg), TCP1 (500 mg/kg), TCP2 (1000 mg/kg) respectively. All the extracts and methotrexate were started on day 1 and continued till day 12 via per oral route once in a day. Phytochemical study of all the extracts was done. Body weight, volume of left paw, hemoglobin (Hb); and inflammatory markers *viz.* Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP) and Rheumatoid Factor (RF) were measured. Higher doses of aqueous extract (500 mg/ kg), dichloromethane extract (100 mg/kg), guduchi satva (1000 mg/kg) and low dose of hydroalcoholic extract (10 mg/kg) showed better improvement in paw volume and reduced inflammatory markers as well as improved hemoglobin level. All the *Tinospora cordifolia* extracts showed antiarthritic and antirheumatoid activity. *Tinospora cordifolia* extracts reduced inflammation and improved RA associated anemia.

KEYWORDS

Tinospora cordifolia, Rheumatoid Arthritis, Complete Freund's Adjuvant, Methotrexate, Guduchi Satva

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease of unknown etiology causing persistent inflammatory synovitis which leads to cartilage damage, bone erosions and subsequent changes in joint integrity. RA is associated with high costs and, if not treated appropriately, significantly reduces life expectancy.

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Drug therapy for RA rests on two principal approaches: symptomatic treatment with non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs). NSAIDs only interfere with a small segment of the inflammatory cascade but do not interfere with the underlying immuno-inflammatory events or retard joint destruction. By contrast, DMARDs 'modify' the disease process in all these respect, and, once DMARDs are effective, no further symptomatic therapies are needed. DMARDs have range of adverse effects including hepatotoxicity, bone marrow

depression, retinopathy and many more. So its need of the day that novel approaches with minimal adverse effects should be found. *Tinospora cordifolia* commonly known as galo, guduchi, giloy or amrita, is widely used in veterinary and folk medicine/ayurvedic system of medicine for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, anti-allergic and anti-diabetic properties. The plant is used in ayurvedic system as "Rasayanas" to improve the immune system and the body resistance against infections. Aqueous extract of it has shown anti-inflammatory and immunosuppressive effect.[1,2] It also showed significant reduction in carrageenan-induced acute inflammation in albino rats.[3] Alcoholic extract of *T. cordifolia* showed anticancer effect on the proliferation and myeloid differentiation of bone marrow precursor cells in a tumor-bearing host.[4] Dichloromethane extract of it showed antineoplastic action in Ehrlich ascites carcinoma bearing mice and cytotoxic effects on Cultured HeLa Cells.[5,6]. Guduchi satva/Galo satva is the official ayurvedic preparation of *Tinospora cordifolia* but least data is available. Till the date, no comparison is available between various extracts of *Tinospora* and guduchi satva. Based upon all this review of ethnomedicinal and pharmacological actions of *Tinospora cordifolia*, we tried to evaluate its antiarthritic activity in experimental rheumatoid arthritis.

MATERIAL AND METHODS

Drugs and Chemicals

Complete Freund's Adjuvant (CFA) (Sigma Aldrich, St. Louis, USA), Methotrexate (Intas Pharmaceuticals, Gujarat, India), Dichloromethane (Sisco Research Laboratories Pvt. Ltd., Mumbai, India), RA factor kit (Span Diagnostics, Gujarat, India), CRP kit (Spinreact Company, Kolkata, India), were purchased.

Collection of the Plant

Fresh stems of *T. cordifolia* were collected from the botanical garden of K. B Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India (June to August). Plant was authenticated by Taxonomist: Dr. Hitesh Solanki, Dept. of Botany, University School of Sciences, Ahmedabad, Gujarat, India. Herbarium specimen (PH/09/0015) was preserved in the Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, for future reference.

Preparation of Extracts

From the shadow dried stems of *T. cordifolia*, coarse powder was made using ball mill. Four extracts *viz.* aqueous extract, hydroalcoholic extract and dichloromethane extract were prepared from powdered stems and guduchi satva was prepared from fresh stems.

Preparation of Aqueous Extract (TCA)

Aqueous extract was prepared using hot maceration method. Powder was boiled in distilled water in 1:3 ratios at 100 ^{0}C for 30 min with gradual shaking. After 30 min, the mixture was filtered and the filtrate was evaporated to dryness at 100 ^{0}C on water bath in a tared flat-bottomed petri dish with occasional shaking. Residue was weighed (yield 16 % w/w).

Preparation Hydroalcoholic Extract (TCH)

Powder was defatted with petroleum ether and then extracted with mixture of ethanol (95% V/V) and distilled water (80:20) using a Soxhlet continuous extraction apparatus. The filtrate was evaporated to dryness at 100 ^{0}C on water bath in a tared flat-bottomed petri dish with gradual shaking, and residue was weighed (yield 13.6 % w/w).

Preparation Dichloro Methane Extract (TCD)

Powder was defatted with petroleum ether followed by chloroform and finally extracted with dichloromethane using a Soxhlet continuous extraction apparatus for 1 week. The filtrate was evaporated to dryness at 100 ^{0}C on water bath in a tared flat-bottomed petri dish, and residue was weighed (yield 2.4 % w/w).

Preparation of Guduchi Satva (TCP)

The fresh stems of *Tinospora* were cut and crushed. Crush was socked in distilled water in sufficient amount for 8 hours. It was properly macerated and filtered with nylon cloth. The

filtrate was kept to allow the starch to settle down the supernatant was decanted and the sediment was further washed with fresh distilled water till it becomes whitish. Finally, it was dried and residue was weighed (yield 10.2 % w/w).

Preliminary Phytochemical Screening

Phytochemical tests, for presence of alkaloids (Dragendorff's test), phenols (Folin-ciocalteu reagent and $FeCl_3$ test), reducing sugars and non-reducing sugars (polysaccharides) (Molisch's and Fehling test), tannins (gelatin and lead acetate test), flavonoids (Shinoda test and Fluroscence test), terpenoids and steroids (Liberman Buchardt test and Salkowski test) were performed for all extracts.[7]

Animals

Female albino *Wistar* rats (body weight: 170 to 200 g) were used. The animals were acclimatized to standard condition of temperature (20 to 22˚C) with relative humidity (30 to 70 %) and 12-hour alternate light & dark cycle in poly propylene cages. Animals were kept on free access to food and water *ad libitum* during the course of experiment. All the animals were maintained in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The protocol (KBIPER/2009/131) was approved by Institutional Animal Ethics Committee (IEAC) - K. B. Institute of Pharmaceutical Education & Research, Gandhinagar, Gujarat, India, under CPCSEA guidelines.

Induction of RA using Complete Freund's Adjuvant (CFA)

Animals were divided in 11 groups each containing 3 animals. RA was induced by method as described by Pearson & Wood.[8] Briefly, all the animals received 0.2 ml CFA (contained 10 mg lyophilized killed *mycobacterium tuberculosis* suspended in mixture of 8.5 ml paraffin oil and 1.5 ml of mannide monooleate) in subplantar region of left paw on day 0 except Group I (normal control). Group II (disease group) received only CFA. Group III (standard group) received methotrexate (0.25 mg/kg). Group IV to XI received various extracts *viz.*, TCA1 (50 mg/kg), TCA2 (500 mg/kg), TCH1 (10 mg/kg), TCH2 (100 mg/kg), TCD1 (10 mg/kg), TCD2 (100 mg/kg), TCP1 (500 mg/kg) and TCP2 (1000 mg/kg) respectively. All the extracts and methotrexate were started on day 1 and continued till day 12 via per oral route once in a day. From day 13 to 21, the animals did not receive any drug or extract. Hydroalcoholic extract **(**TCH) was dissolved in water. Aqueous extract (TCA), guduchi satva (TCP) and methotrexate were suspended in distilled water using carboxy methyl cellulose (CMC) (1% W/V). Dichloromethane extract (TCD) was suspended using 0.5 % Tween 20 aqueous solution.

Table 1: Groups and Treatments for Anti-Rheumatic Activity in CFA Induced RA

Sr. No	Group	Treatment
I	CONTROL	Saline
II	CFA (Disease)	CFA (0.2 ml s. c, oid) (day 0 only)
III	MTX (Standard)	CFA (0.2 ml s. c, oid) +methotrexate (0.25 mg/kg)
IV	TCA1	CFA (0.2 ml s. c, oid) + TCA (50 mg/Kg)
V	TCA2	CFA (0.2 ml s. c, oid) + TCA (500 mg/Kg)
VI	TCH1	CFA (0.2 ml s. c, oid) + TCH (10 mg/kg)
VII	TCH2	CFA (0.2 ml s. c, oid) +TCH (100 mg/kg)
VIII	TCD1	CFA (0.2 ml s. c, oid) + TCD (10 mg/kg)
IX	TCD2	CFA (0.2 ml s. c, oid) + TCD (100 mg/kg)
X	TCP1	CFA (0.2 ml s. c, oid) +TCP (500 mg/kg)
XI	TCP2	CFA (0.2 ml s. c, oid) + TCP (1000 mg/kg)

Parameters

Bodyweight (gms) and volume of left paw (ml) were measured on day 0, 5, 12 and 21. Paw volume was measured using mercury plethysmometer. On day 21, blood was collected from retro orbital area.

It was centrifuged using cooling Remi centrifuge and serum was separated for estimation of hemoglobin and inflammatory markers. Hemoglobin (Hb) (gm %) was measured using automatic single drop hemoglobinometer.

Inflammatory markers, Erythrocyte Sedimentation Rate (ESR) (mm/hr) (Westergren's method), C-ReactiveProtein (CRP) (mg/L) and Rheumatoid Factor (RF) (IU/L) were measured.[9,10]

Statistical Analysis

Data were expressed as mean ± SEM (n=3). $P<0.05$ was considered as statistically significant. One-way Analysis of Variance (ANOVA) was carried out followed by post hoc Tukey test. Statistical analysis was performed using Sigma Plot for Windows version 12 software developed by 2011 Systat Software, Inc.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Aqueous extract (TCA) and guduchi satva (TCP) showed the presence of reducing and non-reducing sugars. Hydroalcoholic extract (TCH) showed the presence of reducing sugars and terpenoids; and in dichloromethane extract (TCD), alkaloids and terpenoids were present.

Body Weight (gms)

Body weight in disease animals was significantly lower as compared to control on day 5, 12 and 21. Significant difference in body weight was observed in MTX (standard) and all TC extracts treated groups except TCD2 and TCP2 as compared to disease group (day 5).

There was significant difference in MTX and all TC extracts except TCP1 (day 12). Significant difference was observed in MTX, TCA1, TCA2, TCH1 and TCH2 on day 21 ($P<0.05$) (Table 2).

Left paw Volume (mL)

Volume of left paw in disease animals was significantly higher as compared to control on day 5, 12 and 21. Significant difference was observed in MTX and all TC extracts treated groups except TCA1 and TCD1 treated groups as compared to disease group on day 5. There was significant difference in MTX and all TC extracts (day 12). There was significant difference in MTX, TCA2 and TCP2 as compared to disease group on day 21 ($P<0.05$).

Hemoglobin and Inflammatory Markers (ESR, RF factor and CRP)

Hemoglobin was significantly lower in disease animals as compared to control. ESR, RF factor and CRP were significantly higher in disease animals as compared to control. MTX and all the TC extracts improved Hb (except TCA1 group), ESR and RF levels significantly decreased in all TC extracts treated animals. MTX and all TC extracts improved CRP level (except TCD1 and TCP1) as compared to disease ($P<0.05$).

Table 2, 3 and 4: Data expressed as mean ± SEM (n=3). * indicates significant difference from normal control; # indicates significant difference from disease control; One-way ANOVA followed by post Tuckey test ($P<0.05$).

From all above data, all the extracts in both the doses showed more or less beneficial effect in CFA induced arthritis. To summarize, aqueous extracts (TCA) improved body weight, left paw volume (not low dose i. e (50 mg/Kg) on day 5 and 21), Hb (no change in low dose), ESR, RF and CRP levels. Amongst hydroalcoholic extracts (TCH), both doses showed equal improvement in all parameters. In dichloromethane extracts (TCD), high dose was more effective than low dose as low dose (10 mg/kg) didn't improve CRP level. While comparing guduchi satva (TCP) doses, low dose (500 mg/kg) didn't improve left paw volume and CRP level on day 21. All these results show that higher doses of aqueous extract (500 mg/ kg), dichloromethane extract (100 mg/kg), guduchi satva (1000 mg/kg) and low dose of hydroalcoholic extract (10 mg/kg)

Table 2a: Effect of TC extracts on body weight (gms)

Sr. No	Groups	Day 0	Day 5	Day 12	Day 21
1	CONTROL	203.33±3.33	216.67±6.01	218.33±6.66	226.66±3.33
2	CFA(Disease)	196.66±4.40	151.66±6.01*	133.33±6.00*	156.67±6.67*
3	MTX(Standard)	205±2.89	190±2.88#	186.67±3.33#	193.33±1.66#
4	TCA1(50 mg/kg)	205±2.89	200±5.77#	186.66±1.66#	208.33±6.66#
5	TCA2(500 mg/kg)	193.33±4.40	190±5#	171.66±3.33#	193.33±4.40#
6	TCH1(10 mg/kg)	203.33±6.66	201.66±6.01#	200±10#	205±15#
7	TCH2(100 mg/kg)	203.33±4.40	205±8.66#	200±11.55#	200±8.66#
8	TCD1(10 mg/kg)	188.33±1.66	191.66±6.01#	193.33±6.67#	186.66±3.33 NS
9	TCD2(100 mg/kg)	185±2.89	170±2.88NS	178.33±4.41#	183.33±6.66 NS
10	TCP1(500 mg/kg)	194±3.22	187.67±3.93#	161.67±2.03NS	169±0.577 NS
11	TCP2(1000 mg/kg)	188.333±4.91	175.67±4.70 NS	172.67±2.91#	176±1.528 NS

Table 2b: Effect of TC extracts on left paw volume (mL)

Sr. No	Groups	Day 0	Day 5	Day 12	Day 21
1	CONTROL	0.93±0.037	0.91±0.049	0.92±0.036	0.93±0.046
2	CFA (Disease)	0.98±0.033	1.55±0.058*	1.72±0.055*	1.45±0.029*
3	MTX (Standard)	1.05±0.028	1.12±0.031#	1.07±0.030#	1.11±0.01#
4	TCA1(50mg/Kg)	1.1±0.040	1.603±0.14 NS	1.3±0.045#	1.49±0.097 NS
5	TCA2(500 mg/kg)	1.056±0.006	1.173±0.041#	1.34±0.045#	1.17±0.090#
6	TCH1(10 mg/kg)	1.016±0.052	1.18±0.0115#	1.23±0.043#	1.45±0.029 NS
7	TCH2(100 mg/kg)	1.043±0.064	1.187±0.0318#	1.23±0.015#	1.31±0.018 NS
8	TCD1(10 mg/kg)	1.01±0.021	1.32±0.140 NS	1.37±0.088#	1.35±0.015 NS
9	TCD2(100 mg/kg)	1.01±0.037	1.16±0.0115#	1.34±0.061#	1.317± 0.044 NS
10	TCP1(500 mg/kg)	0.89±0.038	1.157±0.054#	1.273±0.0371#	1.257±0.023 NS
11	TCP2(1000 mg/kg)	0.913±0.038	1.0167±0.032#	1.037±0.0133#	1.103±0.024#

Table 3: Effect of TC extracts on Hb and inflammatory markers (ESR, RF factor and CRP)

Sr. No	Groups	Hb (gm %)	ESR (mm/hr)	RA (IU/mL)	CRP (mg/L)
1	CONTROL	12.73± 0.56	4±1.16	3.4±0.67	2.67±0.79
2	CFA (Disease)	8.37±0.49*	15.33±0.89*	27.47± 2.69*	10.73±1.24$^{\#}$
3	MTX (Standard)	12.37±0.57$^{\#}$	6±1.16$^{\#}$	7.63±0.38$^{\#}$	3.5±0.35$^{\#}$
4	TCA1(50 mg/Kg)	10.77±0.65^{NS}	4.67±0.67$^{\#}$	2.1±0.40$^{\#}$	6.53±0.83$^{\#}$
5	TCA2(500 mg/Kg)	11.83±0.38$^{\#}$	8±0.58$^{\#}$	3.07±0.7$^{\#}$	6.83±0.23$^{\#}$
6	TCH1(10 mg/kg)	12.2±0.68$^{\#}$	9.67±1.86$^{\#}$	3.37±0.67$^{\#}$	5.83±0.8$^{\#}$
7	TCH2(100 mg/kg)	13.1±0.23$^{\#}$	10±0.58$^{\#}$	4.97±0.41$^{\#}$	5.43±0.73$^{\#}$
8	TCD1(10 mg/kg)	11.6±0.44$^{\#}$	9.67±0.88$^{\#}$	6.13±0.71$^{\#}$	8.73±0.7^{NS}
9	TCD2(100 mg/kg)	11.9±0.68$^{\#}$	7±0.58$^{\#}$	4.97±0.45$^{\#}$	6.97±0.09$^{\#}$
10	TCP1(500 mg/kg)	14.07±0.74$^{\#}$	10.33±0.88$^{\#}$	11.37±0.43$^{\#}$	7.37±0.41^{NS}
11	TCP2(1000 mg/kg)	13.43±0.27$^{\#}$	7.67±0.33$^{\#}$	8.13±0.35$^{\#}$	5.23±0.49$^{\#}$

showed improvement in paw volume and reduced inflammatory markers as well as improved hemoglobin level. *T. cordifolia* is reported to have immunomodulatory effect in various animal experiments.[11,12] Furthermore, previous study also showed anti-inflammatory effect of *T. cordifolia.*[13] Immunomodulatory compounds have been isolated from it.[14] The plant has also shown anti-metastatic and anti-angiogenic effect.[15,16] These also studies support our study. In phytochemical screening of various extracts, alkaloids in dichloromethane extract; reducing sugars in aqueous extract, hydroalcoholic extract and galo satva; terpenoids in hydroalcoholic extract and dichloromethane extract; non-reducing sugars (polysaccharides) in aqueous extract and galo satva were found to be present. Possibly, these constituents may be responsible for antirheumatoid activity.

CONCLUSION

All the *Tinospora cordifolia* extracts showed antiarthritic and antirheumatoid activity. Aqueous extract (500 mg/ kg), dichloromethane extract (100 mg/kg), guduchi satva (1000 mg/kg) and hydroalcoholic extract (10 mg/kg) reduced inflammation and improved RA associated anemia.

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