Rakesh Sarma M et al /J. Pharm. Sci. & Res. Vol. 11(11), 2019, 3579-3586

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Anti-inflammatory and Anti-granuloma effect of the extract of the leaf of *Terminalia arjuna* (Roxb.) Wight & Arn

Rakesh Sarma M [1], Balanehru Subramanian*[1, 2], Tamilmaran P [2], Ramakrishnan G [2]

[1]*Central Inter-Disciplinary Research Facility (CIDRF), Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, 607402, India*

[2] *Center for Animal Research, Training and Services (CAReTS), Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, 607402, India*

Abstract

Background: *Terminalia arjuna* (Roxb.) Wight & Arn belonging to the family of Combretaceae is widely used in Indian System of Medicine. Studies with *Terminalia arjuna* leaf extract have been reported for their antimicrobial and anti-oxidant potential *in vitro*. **Aim:** The aim of the present study was to evaluate the anti-inflammatory and anti-granuloma potential of methylene chloride methanolic extract of leaf of *Terminalia arjuna* (MMTL).

Objective: Carrageenan-induced paw edema and cotton pellet-induced granuloma (CPIG) in Wistar rats, were used as a model systems by measuring the paw edema volume and granulomatous tissue weight as indicators of biological activity. **Methodology:** In carrageenan-induced animal model, MMTL at two doses of 200 and 400 mg/k; standard drug, meloxicam at a dose of 2 mg/kg body weight were administered orally 30 min before carrageenan injection in sub-plantar region of the left hind paw of the rat. The same drug treatment protocol was followed in cotton pellet-induced granuloma model except that treatment continued for seven consecutive days while cotton pellet was implanted on first day of dosing. Changes in hematological parameters and oxidative stress markers were measured. Granulomatous tissues were excised for further histopathological evaluation.

Results: Pretreatment of carrageenan-induced group with MMTL at 200 and 400 mg/kg of body weight was found to reduce paw edema volume by 38.05% and 64.95% respectively the granulomatous tissue weight was 115.33 mg and 73.67 mg. Hematological analysis revealed that in MMTL pre-treatment animals were decreased the level of WBC and platelets with an increase in Hb and RBC level. MMTL extract pre-treatment was found to reduce the amount of nitrite and LPO increasing the levels of anti-oxidants enzymes, namely SOD, GSH and GPx. Histopathology results revealed that MMTL treatment reduced the inflammatory cells accumulation with respective to CPIG-treated group.

Conclusion: These results indicate that MMTL extract has therapeutic potential anti-inflammatory and anti-granuloma efficacy.

Key words: anti-inflammation, anti-granuloma, *Terminalia arjuna*, Leaf extract, natural product, Methylene chloride methanol.

Abbreviations

MMTL - Methylene chloride Methanolic extract of leaf of *Terminalia arjuna*.
CMC - Carboxy methyl cellulose
OECD - Organisation for Economic Co-operation and Development
CPIG - Cotton pellet induced granuloma
SOD - Superoxide dismutase
GPx- Glutathione peroxidase
GSH - Reduced glutathione
LPO – Lipid per oxidation
GHS - Globally Harmonised System
WHO-World Health organization
RBC- Red Blood Cell
WBC-White Blood Cell
Hb- Hemoglobin
PLT- Platelets

Acknowledgements

The authors acknowledge the Center for Animal Research, Training and Services (CAReTS), Sri Balaji Vidyapeeth (Deemed to be University) for providing technical and infrastructure support.

1. INTRODUCTION

An inflammatory disease has multifactorial etiology therefore its management is more complicated, expensive and time consuming. Current pharmacological agents exert an anti-inflammatory action, their adverse effects and withdrawal syndromes represent significant limitations to prolonged exposure.Large number of negative results in clinical trials warrants the need for newer chemical entities for the treatment of inflammatory diseases. With this milieu, an attempt was made in the present study to identify adjuvant therapeutic strategy for treating inflammatory diseases.

Herbal medicine has gained considerable attention, and its therapeutic interventions for different metabolic disorders and diseases, including inflammatory diseases, have already been recognized. The World Health Organization strongly recommends herbal based traditional medicine, because of their easy availability, affordable cost and less adverse effect [1]. Indian medicinal herbs have served long as nutraceuticals, dietary supplements and

pharmaceutical intermediates [2]. Several of these medicinal herbs and their phytoconstituents have been used for treatment and management of inflammatory diseases. However, many medicinal herbs usage lacks proper clinical evidence. Therefore, the present was aimed to evaluate the ethnomedical value of a traditional herb, *T.arjuna* for the treatment and management of inflammatory diseases.

T.arjuna, a well-known medicinal plant, holds an ethnomedical importance in Indian system of medicine. It has been reported that bark contains cardio-protective [3] and antibacterial effect [4], leaves exhibits antioxidant and antimicrobial properties [5, 6]. The various active phytoconstituents present in *T.arjuna* offered some protection against inflammation [7; 8]. The leaf extract of *T.arjuna* has been characterized for the presence of phytoconstituents such as polyphenols, glycosides and alkaloids using GC-MS and LC-MS analysis (data not shown). Based on the above findings, natural products and their active constituents with anti-oxidants properties might control the inflammation and their associated pathogenesis. In this study, we have evaluated the anti-inflammatory activity of MMTL (Methylene chloride Methanolic extract of *Terminalia arjuna*) against acute-phase of inflammation (carrageenan-induced paw edema) and chronic phases of inflammation (cotton pellet-induced granuloma) in Wistar rat model.

2. MATERIALS AND METHODS

Chemicals

Meloxicam was purchased from Pharmacy, Mahatma Gandhi Medical College and Research Institute (MGMCRI), Puducherry. λ-Carrageenan was purchased from Sigma Aldrich, USA. All other chemicals and solvents were of analytical grade purchased from Merck, AIC International, Chennai. Pleythysmometer instrument was purchased from Orchid Scientific, India.

Collection and preparation of plant material

Leaves of *T.arjuna* were collected from in and around Puducherry to a radius of 40 kilometers recorded with GPS identification. Plant material was certified by the Department of Agriculture, Puducherry. Freshly collected leaves were washed thoroughly in slow running tap water to remove adhered debris. After final rinse with distilled water, the leaves of*T.arjuna*were shade dried separately for 14 days. Dried leaves were ground to a particle size of approximately 3.5mm, ground by using pestle and motor. The powdered material was stored at room temperature in air tight zip lock bags until use [9].

Extract preparation

The extraction procedure [10] was performed with slight modification. Sixty grams of the leaf powder was macerated in 1.5 L of methylene chloride-methanol mixture (1:1). The mixture was then kept in a shaker in the laboratory condition for seven days. During this period, extract mixture was stirred twice daily. The mixture was then filtered through metallic sieves (500 μm) and a layer of cotton and Whatmann no.1filter paper. The final filtrates were then down concentrated using a rotavapor (Büch-R-124, USA). The concentrated extract was transferred to watch glass plates and kept overnight in the water bath at 50°C for evaporating the solvent present in the extract. The total yield was calculated by Dry wt. / Wet wt. * 100. The concentrated methylene chloride methanolic extract of *T. arjuna* ("MMTL") was stored at 4°C until further use.

Experimental design.

Female Wistar rats (140-190g) and Male Wistar rats (200-220g) were used in the present study. Animals were housed in a well ventilated polypropylene cage. A 12-h light/12-h dark artificial photoperiod was maintained at 22 to 24 °C ±1 temperature and relative humidity at 30-70 %. Animals had free access to pelleted feed (commercially available standard pellet M/s. VRK India) and reverse osmosis purified water ad libitum. All the experimental procedures were approved by the Institutional Animal Ethics Committee (Reg. No. 05/IAEC/MG/06/2018-I and 04/IAEC/MG/2016-2). Each treatment group consisted of six animals (n= 6). The test samples (including MMTL, and meloxicam) were orally administered to animals in 0.5% carboxy methyl cellulose (0.5% CMC). Control group animals received 0.5% CMC only.

Acute oral toxicity

Acute oral toxicity study was performed according to the OECD (The Organisation for Economic Co-operation and Development) test guideline 423- Acute Oral Toxicity – Acute Toxic Class Method. Healthy young adult nulliparous and non-pregnant Wistar albino female rats were used for this study. Animals were kept in their cages for 5 days prior to dosing for acclimatization to the laboratory conditions. Prior to the study animals were fasted for overnight and then 3 - 4hrs post administration of MMTL extract. This experiment was conducted with step wise procedure. 300 mg/kg b.wt was selected as the starting dose. Each step dosed (300 mg/kg b.wt (Step I, II) and 2000mg/kg (Step III & IV) with three animals by gastric intubation. Lethality and abnormal clinical signs were observed after test item administration. Clinical signs were observed on the day of dosing at 30 min, 1hr, 2hr and 4hr and until for 14 days of observation period. The time interval between the administrations of dose to animals was determined by observing the survival and severity of toxic signs of the treated animals during the first 48 hours of dosing. Body weights were recorded just prior to dosing and thereafter once in a week till completion of the experiment. Gross pathological changes were also observed at the end of observation period [11].

Carrageenan induced paw edema in rats

24 Wistar male rats between the ranges of 200-220 gm of body weight were selected and acclimatized for a period of five days to the laboratory condition. After acclimatization, animals were randomized into four groups (Group I –IV) consisting of six animals per group. Group I animals received vehicle (0.5% CMC); Group II animals received Meloxicam 2 mg/kg p.o; Group III animals

received MMTL 200 mg/kg, p.o ; Group IV animals received MMTL 400 mg/kg p.o. After one hr of the drug administration, 0.1 ml of 1% carrageenan was injected into the sub plantar region of the left hind paw of all experimental rats. Drug dosage was adjusted based on the respective body weight of animals. The present experiment was carried out to determine the effectiveness of MMTL extract dose dependently alleviating the inflammation of carrageenan on paw edema changes in left hind paw of rat using water displacement method (Pleythysmometer, Orchid scientific, India). The values of body weight, paw edema and % of protection edema volume were expressed Mean ± SEM.

The percentage rise in paw volume was calculated using the formula [12].

% Rise = (Vt - V0)/V0 X 100

Where, Vt = Paw volume at time t; V0 = Initial paw volume.

Cotton pellet induced granuloma in rats

Five groups of Wistar albino male rats (n = 6) was used in the study. Group I served as normal control, Group II served as diseased control; Group III was administered with standard drug Meloxicam 2mg/kg; Group IV, and V were treated with MMTL at doses of 200 and 400 mg/kg, respectively. 30 minutes after administration of drug/vehicle, the animals were anesthetized using ketamine (50mg/kg) and Xylazine (5mg/kg) and a sterile cotton pellet weighing (10 ± 1 mg) saturated with normal saline was implanted subcutaneously bilaterally below the axilla. Drug/vehicle treatments were continued for the duration of seven days. On the 8th day, terminal blood collection was carried out and the serum was separated by centrifugation at 3000 rpm at 10 min and used for biochemical estimation. At end of the experiment, all animals were euthanized using isoflurane and then wet weight of cotton pellets was collected. The pellets were then dried overnight at 60°C until a constant weight was recorded for two consecutive recordings. The difference between the initial and post implantation weight was considered to be the dry weight of the granuloma tissue. The percentage of inhibition of exudate and granuloma tissue formation was determined [13].

Antioxidant analysis

To evaluate the effect of MMTL extract in subduing the oxidative stress, the levels of Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Reduced glutathione (GSH), Lipid peroxidation (LPO) and Nitrite levels was estimated in the hepatic tissue prepared using 10% potassium chloride [14].

Histopathological analysis

The excised granulomatous tissues removed from cotton pellet-induced granuloma (CPIG) rats was fixed in 10% neutral buffered formalin solution for a period of 24 h and later on processed for paraffin embedding. Tissue were sectioned and made a cut at 4–5 µm thickness, flattened and sticked to the slides. Subsequently wax was removed in tissue sections (4 µm) by gradual washings in xylene followed by hydration with various concentrations of alcohol in automatic tissue processor (Thermo Scientific, USA). Haematoxylin & Eosin staining was performed on all samples for pathological examination. The sections were examined using light microscope (Carl Zeiss Axio, Germany) [15].

Statistics

Data was expressed as Mean±SEM (n=6). Statistical analysis was carried out using Graph Pad Prism 5.0, USA. Difference between groups was analyzed by One-way ANOVA followed by Tukey multiple comparison as post hoc test. $p< 0.05$ was considered statistically significant.

3. RESULTS

Acute oral toxicity study

Data was analysed according to the OECD test guideline 423: Acute toxic class method (adopted 17th December 2001). Oral administration of highest dose 2000 mg/kg of MMTL extracts do not exhibit any mortality or any signs of toxicity during the observation period. There was no decrease in body weight and no gross pathological lesions observed in the experimental animals (Table 1). The LD_{50} of "MMTL extract" was found to be greater than 2000 mg/kg when administered orally to Wistar Rats. The extracts were found to be safe at the highest dose of 2000 mg/kg; five and ten fold dilutions of the highest dose were selected for anti-inflammatory and anti-granuloma activities.

Carrageenan-induced paw edema in rats

Administration of carrageenan in the sub plantar region of the left hind limb produced paw edema in all the experimental groups throughout the observation period (1, 2, 3, 4, and 5 h) Fig 1. Treatment with MMTL extract at 400 mg/kg dose showed a significant ($p<0.01$) inhibition of carrageenan-induced hind paw edema with maximum percentage of protection (64%), at 5th hr after carrageenan injection (Table 2). The standard drug meloxicam at 2 mg/kg produced significant reduction ($p<0.01$) in paw edema volume from 3rd hr to 5th hr when compared to carrageenan control groups. The reductions in the paw volume, edema volume by MMTL extract (200 mg/kg and 400 mg/kg) treated groups are shown in table 3 and 4.

Effect of body weight and organ weight in the CPIG

The body weight of cotton pellet animals with induced granuloma were measured at Day 0 and Day 8 and the organ weight of spleen and thymus were measured after necropsy as represented in Table 5. No significant difference in the body weight was observed when compared between day 0 and day 8. Pretreatment of MMTL extract showed significant reduction ($p < 0.05$) in spleen weight but there was no significant weight reduction in the thymus when compared to CPIG control groups.

Effect of MMTL on granuloma weight in the CPIG

Granulomatous tissue weights were recorded in cotton pellet-induced granuloma rats. Pretreatment with MMTL

extract (200 and 400 mg/kg) showed remarkable difference from the wet, dry granuloma weight and the results are represented in Table 6. The difference in the size of granulomatous tissue formation after excision from the CPIG is shown in Fig 2. Pretreatment with MMTL extract showed significantly ($p < 0.01$) decreased granulomatous tissue weight when compared to CPIG control groups. The MMTL extract of 400 mg/kg was found to be equally potent as that of meloxicam.

Effect of MMTL on hematological changes in the CPIG rats

Table 7 depicts the Blood parameter (WBC, RBC, Hb and PLT) changes that observed in CPIG rats. Pretreatment with MMTL extract showed dose-dependent decrease WBC and PLT counts with significant ($p < 0.01$) increase in RBC and Hb levels when compared to CPIG control group.

Effect of MMTL on oxidative stress and inflammatory markers in the hepatic tissue of CPIG rats

Pretreatment with MMTL extract showed dose-dependent increase in the level of antioxidant enzymes SOD, GPx and GSH with a significant ($p<0.01$) reduction of LPO and nitrite level when compared with that of CPIG control group. (Table 8)

Effect of MMTL on histopathological changes in CPIG rats

Granuloma tissue sections that comprises from CPIG group possess inflammatory cells such as neutrophils, lymphocytes, macrophages and few plasma cells with proliferating blood vessels were noted. Whereas tissues section from pretreatment with Meloxicam (2 mg/kg) showed the absence of necrosis and reduced inflammatory cells infiltration. Likewise, pretreatment with MMTL extract of 400 mg /kg b.wt showed minimal infiltration by lymphocyte, macrophages, and few plasma cells when compared with CPIG control group. (Fig 3)

Table 1 The effect of MMTL on Body weight

Treatment	A. No.	Sex	Body weight (gm)		
			0th Week	1st Week	2nd Week
TA-Leaf extract 300mg/kg b. wt (Step I)	1	F	144.0	167.0	168.0
	2	F	176.0	197.0	201.0
	3	F	154.0	169.0	172.1
TA-Leaf extract 300mg/kg b. wt (Step II)	4	F	160.0	175.0	181.0
	5	F	169.0	199.0	205.2
	6	F	142.0	160.0	161.5
TA-Leaf extract 2000mg/kg b.wt (Step III)	7	F	189.1	196.0	211.0
	8	F	158.0	161.1	182.1
	9	F	156.3	160.6	175.0
TA-Leaf extract 2000mg/kg b. w (Step IV)	10	F	162.1	180.6	198.2
	11	F	166.4	183.4	195.0
	12	F	156.7	166.8	188.0

A. No. – Animal Number, F – Female, gm – gram, b.wt – Body weight

Table 2 Effect of MMTL on changes in paw volume in carrageenan induced rats

Treatment	0hr (in ml)	0.5hr (in ml)	1 hr (in ml)	2 hr (in ml)	3 hr (in ml)	4 hr (in ml)	5 hr (in ml)
1% Carrageenan	1.00±0.04	1.29±0.14	1.57±0.05	1.77±0.09	1.89±0.03	2.01±0.03	2.13±0.05
Meloxicam 2mg/kg	1.23±0.04	1.49±0.06	1.46±0.03	1.44±0.05	1.44±0.07**	1.45±0.05**	1.41±0.07**
MMTL 200 mg/kg	1.22±0.08	1.51±0.03	1.69±0.07	1.81±0.11	1.76±0.13	1.88±0.04	1.92±0.05
MMTL 400 mg.kg	1.18±0.08	1.43±0.10	1.60±0.09	1.67±0.12	1.63±0.08	1.62±0.11**	1.58±0.08**

The effect of MMTL on paw volume in carrageenan induced rats. Results were expressed in mean ± SEM, (n = 6).
* $p < 0.05$, **$p < 0.01$- compared with 1% Carrageenan using One way ANOVA followed by Tukey as post hoc test.

Table 3 Effect of MMTL on Changes in edema volume in carrageenan induced rats

Treatment	0.5 hr (in ml)	1 hr (in ml)	2 hr (in ml)	3 hr (in ml)	4 hr (in ml)	5 hr (in ml)
1% Carrageenan	0.29±0.11	0.57±0.04	0.76±0.07	0.89±0.03	1.01±0.03	1.13±0.04
Meloxicam 2mg/kg	0.27±0.03	0.24±0.03	0.21±0.03**	0.21±0.08**	0.23±0.05**	0.18±0.08**
MMTL 200 mg/kg	0.29±0.09	0.47±0.09	0.59±0.07	0.55±0.12	0.66±0.06*	0.70±0.08*
MMTL 400 mg.kg	0.25±0.11	0.42±0.12	0.49±0.11	0.45±0.10*	0.44±0.13**	0.40±0.11**

The effect of MMTL on edema volume in carrageenan induced rats. Results were expressed in mean ± SEM, (n = 6). *$p < 0.05$,**$p < 0.01$- compared with 1% Carrageenan using One way ANOVA followed by Tukey as post hoc test.

Table 4 Effect of MMTL on percentages protection in carrageenan induced rats

Treatment	0.5 hr (%)	1 hr (%)	2 hr (%)	3 hr (%)	4 hr (%)	5 hr (%)
Meloxicam 2mg/kg	8.05	58.48	71.78	76.34	77.56	84.17
MMTL 200 mg/kg	0.00	17.54	22.37	38.20	34.65	38.05
MMTL 400 mg.kg	14.94	26.32	35.53	49.44	56.71	64.95

The effect of MMTL on percentage protection in carrageenan induced rats. MMTL – Methylene chloride Methanol *Terminalia arjuna* Leaf extract

Table 5 Effect of MMTL on body weight and organ weight in the cotton pellet induced granulomic animal model

Treatment	B.wt (Day 0) in (gm)	B.wt (Day 8) in (gm)	Spleen in (gm)	Thymus in (gm)
Normal Control	202.17 ±7.58	220.17±8.17	0.98±0.14	0.13±0.02
CPIG	203.00± 4.35	211.83±4.06	1.72± 0.27$^{\#}$	0.18±0.02
Meloxicam 2mg/kg	200.83± 5.15	214.00± 8.42	0.78±0.05**	0.15±0.02
MMTL 200 mg/kg	205.33± 10.64	213.33± 11.54	1.05±0.11*	0.15±0.02
MMTL 400 mg/kg	203.33±5.58	216.33±4.45	1.00±0.06*	0.16 ±0.2

Values are expressed as mean ± SEM, (n = 6).# $p < 0.05$, $^{\#\#}p < 0.01$- comparison between normal control and CPIG, * $p < 0.05$, **$p < 0.01$- comparison between CPIG and drug treated. Statistical Analysis was carried out using One way ANOVA followed by Tukey as post hoc test.

Table 6 Effect of MMTL on granuloma formation and transudation on cotton pellet- induced granuloma formation in rats

Treatment	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg)
CPIG	575.50 ± 4.98	166.00 ± 7.26	409.50 ± 46.10	146.00 ± 7.26
Meloxicam 2mg/kg	308.67 ± 15.34**	67.83 ± 4.28**	240.83 ± 15.86**	47.83 ± 4.28**
MMTL 200 mg/kg	458.67 ± 17.04*	135.33 ± 3.96**	323.33 ± 14.45	115.33 ± 3.96**
MMTL 400 mg/kg	380.50 ± 12.79**	93.67 ± 5.24**	286.83 ± 8.29*	73.67 ± 5.24**

Values are expressed in mean ± SEM, (n = 6). * $p < 0.05$, **$p < 0.01$- compared with CPIG treated using One way ANOVA followed by Tukey as post hoc test.

Table 7 Effect of MMTL on hematological changes in the cotton pellet induced granulomic rats

Treatment	WBC (10^3/uL)	RBC (10^6/uL)	Hb(g/dL)	PLT (10^3/uL)
Normal Control	9.29 ±0.94	11.13± 0.52	14.37 ± 0.43	660.33±11.90
CPIG	15.81 ± 0.88$^{\#\#}$	6.36 ± 0.53$^{\#\#}$	8.70 ± 0.84$^{\#\#}$	984.67 ±81.94$^{\#\#}$
Meloxicam 2mg/kg	8.33 ±1.57**	9.74 ±0.53**	12.17±0.63*	732.50± 21.92**
MMTL 200 mg/kg	10.68 ± 1.19*	8.69± 0.55*	12.25± 1.21*	804.17± 31.63*
MMTL 400 mg/kg	9.86 ± 0.55**	9.46±0.42**	13.33±0.69**	730.00± 27.94**

Values are expressed as mean ± SEM, (n = 6). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$- comparison between normal control and CPIG, *$p < 0.05$, **$p < 0.01$- comparison between CPIG and drug treated. Statistical Analysis was carried out using One way ANOVA followed by Tukey as post hoc test.

Table 8 Effect of MMTL on Antioxidant marker in the cotton pellet induced granulomic rats

Treatment	SOD units/min/mg ptn	GPx nm/min/mg ptn	GSH mcm /g	LPO nm/ mg ptn	Nitrite nm /mg ptn
Normal Control	8.78±0.22	36.12±1.42	5.21±0.35	1.23±0.10	22.21±1.17
CPIG	4.67±0.31$^{\#\#}$	20.84±0.67$^{\#\#}$	2.01±0.16$^{\#\#}$	2.34±0.14$^{\#\#}$	46.99±1.07$^{\#\#}$
Meloxicam (2mg/kg)	6.98±0.23**	38.18±1.28**	5.04±0.13**	0.97±0.05**	28.78±1.24**
MMTL 200 mg/kg	5.81±0.20*	27.43±1.39*	2.91±0.12*	1.06±0.12**	26.26±0.88**
MMTL 400 mg/kg	6.49±0.26**	32.39±2.09**	4.05±0.10**	1.31±0.08**	29.05±0.65**

Values are expressed as mean ± SEM, (n = 6). $^{\#}p < 0.05$, $^{\#\#}$ $p < 0.01$- comparison between normal control and CPIG, $^{*}p < 0.05$, ** $p < 0.01$- comparison between CPIG and drug treated. Statistical Analysis was carried out using One way ANOVA followed by Tukey as post hoc test.

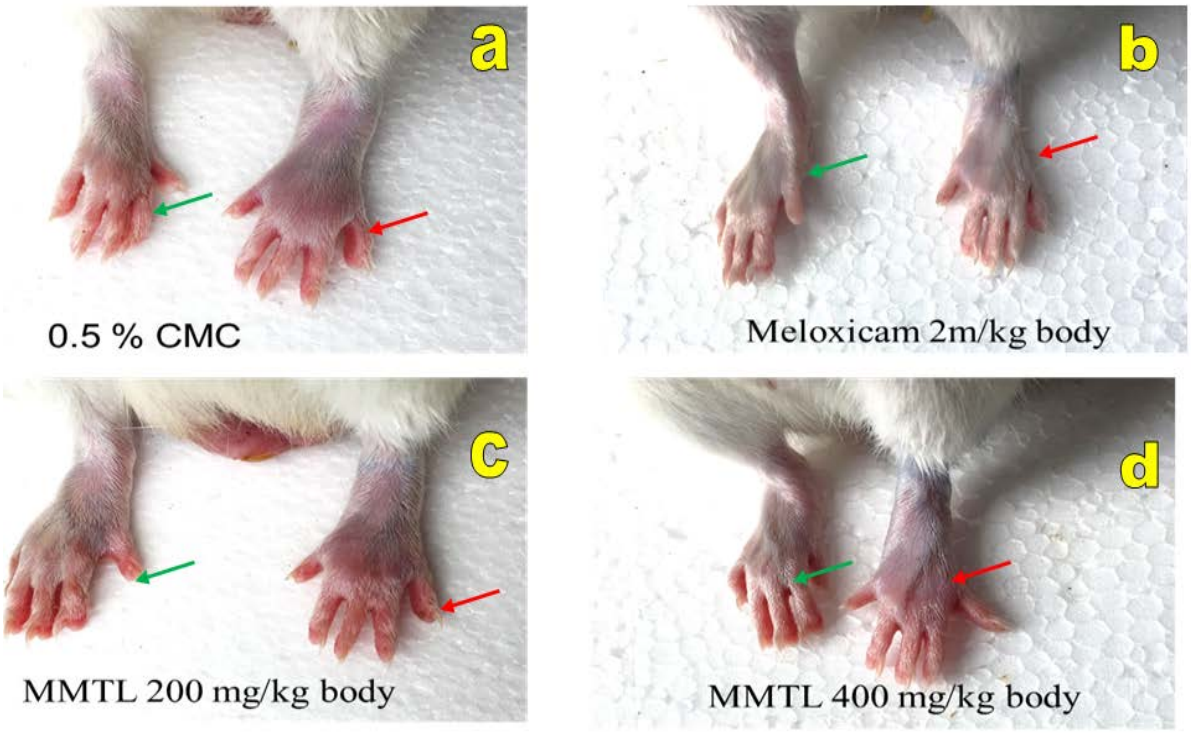

Figure 1 Effect of MMTL on changes in paw edema volume in 1% carrageenan induced inflammation rats.
Red arrow indicates the carrageenan injected Paw, Green arrow indicates non-injected Paw as a control. a) 0.5% CMC b) 0.5% CMC+ meloxicam 2mg/kg c) 0.5% CMC+ MMTL 200 mg/kg d) 0.5% CMC+ MMTL 200 mg/kg.

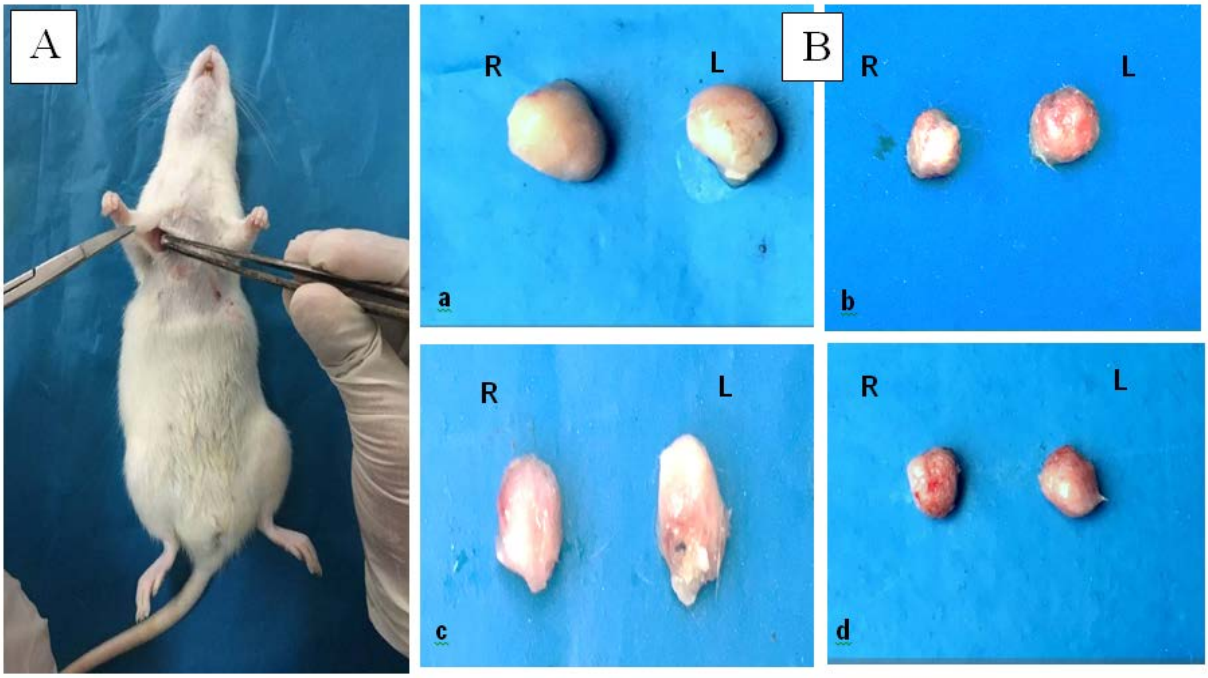

Figure 2
A) Pre-weighed autoclaved cotton pellets implanted. B) Excised cotton pellets with attached granulomatous tissue from all experimental groups
R- Right Axila Cotton, L- Left Axila Cotton. a) 0.5% CMC b) 0.5% CMC+ meloxicam 2mg/kg c) 0.5% CMC+ MMTL 200 mg/kg d) 0.5% CMC+ MMTL 200 mg/kg

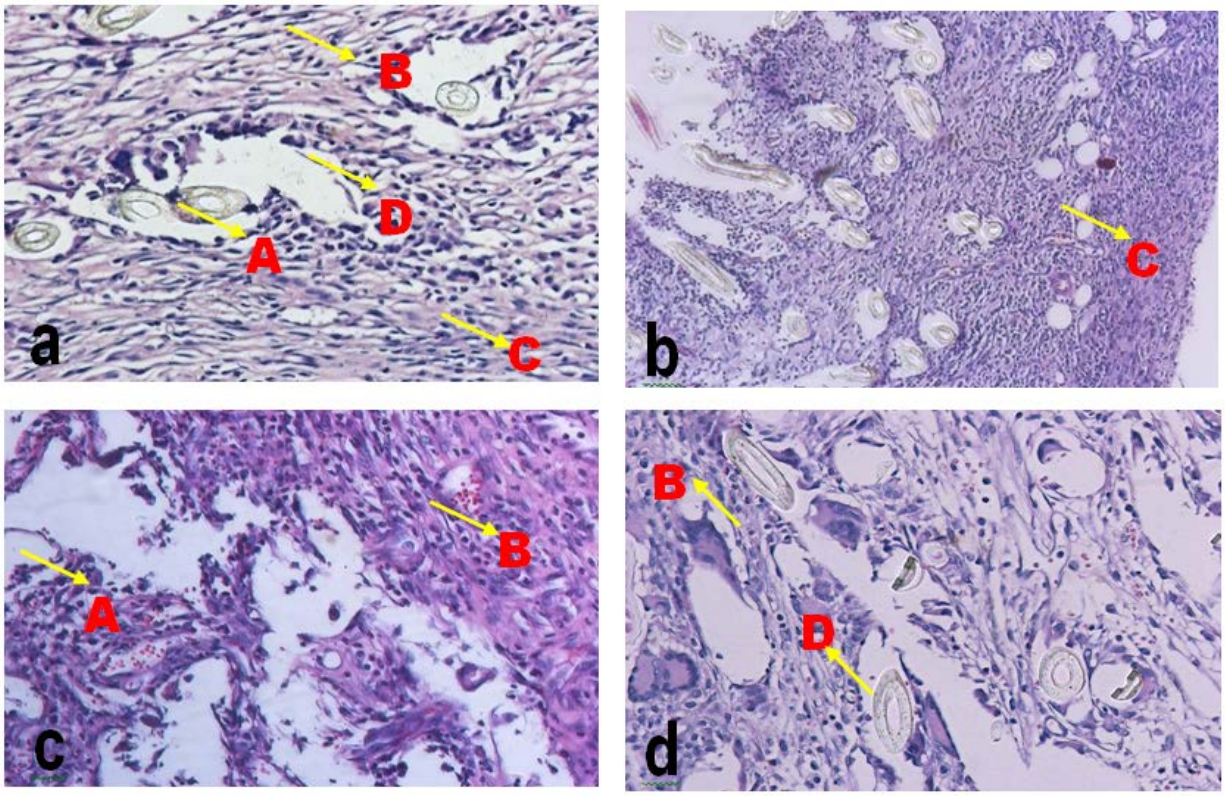

Figure 3 Effect of MMTL on Histopathological changes in cotton pellet induced granuloma

A. Neutrophils, B. Lymphocyte, C. Fibrous tissue, D. Plasma cells. a) CPIG Control group showed fibrous tissues comprising of neutrophils, lymphocytes and few plasma cells with proliferating blood vessels and dense infiltration by acute inflammatory cells. b) Standard group treated with meloxicam revealed fibrous tissues with reduced inflammatory cells. c & d) the rats treated with two different doses of MMTL groups showed the presence of diffusely arranged neutrophils, lymphocytes, macrophages and few plasma cells.

4. DISCUSSION

In the present study, acute oral toxicity of MMTL extract was determined in Wistar rats as per OECD Test guideline 423. The MMTL extract was studied at a maximum dose of 2000 mg/kg b.wt and did not exhibit show any mortality or clinical signs of toxicity during the study. The LD_{50} value of MMTL extract was found to be greater than 2000mg/kg b.wt and classified as category 5 under Globally Harmonised System (GHS) classification.

The carrageenan-induced paw edema is a well- known acute model of inflammation, because the pathophysiological changes following carrageenan administration are similar to clinical condition [16]. In the present study, we observed that carrageenan injection rats showed remarkable elevation of paw edema due to biphasic inflammation which is consistent with the earlier findings [17]. The experimental data showed that MMTL extract presented a dose-dependent inhibition of edema volume after 3rd hr of carrageenan stimuli. i.e. late phase of inflammation. Based on the above findings, the anti-edematous effect of MMTL extract, probably through blocking the release of all mediators of inflammation implicated in the vascular and cellular phases.

In CPIG model, pellet induce granulomatous tissue formation depends on proliferation of macrophages, neutrophils and fibroblasts. In our study, we observed that a remarkable increase of both wet and dry weight granuloma tissue in CPIG control group, indicating the proliferation of inflammatory mediators. Treatment with MMTL extract (200 mg/kg and 400 mg/kg) significantly ($p<0.01$) decrease in the weight of granuloma tissue which indicates that the suppression of proliferative phase of inflammatory response. From these data it is clear that the protective effects observed with MMTL extract against pellet induced inflammation and granuloma formation might be due to the anti inflammatory effect.

A release of platelets and WBC in biological system is part of the defense mechanism against pathogens and other inflammatory stimuli. Here, we also observed that elevated levels of WBC, platelet count in CPIG rats while decrease in Hb and RBC level, which were correlated with report by [14]. Pretreatment with MMTL extract showed restoration of hematological changes to normal level, indicative anti inflammatory potential. These anti inflammatory effects are due to stabilization of reticulo-endothelial system, suppression of leucocytes infiltration and inflammatory mediators release into inflamed area.

A primary measure of oxy-radical damage in tissues is lipid peroxidation (LPO). LPO is an oxidative deterioration reaction is well documented during inflammation [18]. The increase in LPO formation in the hepatic tissue and exudates of pellet granuloma-induced in rats indicate that inflammation was due to the release of autacoids [19; 20]. In the present study, MMTL extract

have been found to decrease LPO and nitrite level while concomitantly increased the SOD, GSH and GPx level which might be due to its ability to neutralize the free radicals in hepatic tissue and exudates of experimental animals. This observation indicates that MMTL extract may function as a free radical scavenger and could be used as a potential agent in treatment of acute and chronic inflammation.

Histopathology findings of excised granulomatous tissue from CPIG control rats showed presence of massive fibro muscular tissues including infiltrated inflammatory cells. These observations along with hematology and biochemical changes confirmed the severity of chronic inflammation. Pretreatment of MMTL extract groups showed mild necrosis and infiltration of inflammatory cells. Based on the above findings, MMTL extract in a dose-dependent manner inhibited the release of inflammatory mediators and is considered as a potent therapeutic agent in inflammation.

5. CONCLUSION

In conclusion, the present data demonstrated that MMTL extract exert beneficial effects against acute or chronic inflammation by reducing edema volume, scavenging free radicals, and activating endogenous antioxidant enzymes. Further, the expression of all inflammatory mediators induced by exudates of acute or chronic inflammation stimuli was also suppressed. Thus, our finding suggests that MMTL could be used as a potential agent in treatment of acute and chronic inflammation.

REFERENCES

1. Palhares, R. M., Drummond, M. G., Brasil, B. D. S. A. F., Cosenza, G. P., Brandão, M. D. G. L., & Oliveira, G. (2015). Medicinal plants recommended by the world health organization: DNA barcode identification associated with chemical analyses guarantees their quality.
2. Lalitha, N., Protecting traditional knowledge in siddha system of medicine. Journal of Intellectual property rights. 2013, 18, 272-282.
3. Dwivedi, S., 2007.*Terminalia arjuna* Wight & Arn - a useful drug for cardiovascular disorders. J Ethnopharmacol. 2,114-129. https://doi.org/10.1016/j.jep.2007.08.003
4. Mandal, S., Patra, A., Samanta, A., Roy, S., Mandal, A., Mahapatra, T.D., Nandi, D.K., 2013. Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties. Asian Pac J Trop Biomed.3, 960-966. https://doi.org/10.1016/S2221-1691 (13) 60186-0.
5. Kumar, V., Sharma, N., Sourirajan, A., Khosla, P. K., & Dev, K., 2018. Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of *Terminalia arjuna* from north-western Himalayas, India. J Tradit Complement Med, 8, 100-106. https://doi: 10.1016/j.jtcme.2017.04.002.
6. Aneja, K. R., Sharma, C., & Joshi, R., 2012. Antimicrobial activity of *Terminalia arjuna* Wight & Arn.: An ethnomedicinal plant against pathogens causing ear infection. Braz J Otorhinolaryngol, 78, 68-74. https://doi.org/10.1590/S1808-86942012000100011.
7. Amalraj, A., Gopi, S., 2017. Medicinal properties of Terminalia arjuna (Roxb.) Wight & Arn.: a review. J Tradit Complement Med. 7, 65-67. https://doi.org/10.1016/j.jtcme.2016.02.003.
8. Hemalatha, T., Pulavendran, S., Balachandran, C., Manohar, B.M., Puvanakrishnan, R., 2010. Arjunolic acid: a novel phytomedicine with multifunctional therapeutic applications. Indian J Exp Biol, 48, 238-247. https://doi.org/10.1016/j.biochi.2013.01.016
9. Mabhiza, D., Chitemerere, T., Mukanganyama, S., 2016. Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernonia adoensis against Staphylococcus aureus and Pseudomonas aeruginosa. Int J Med Chem. 2016, ID–6304163, 1-7. http://dx.doi.org/10.1155/2016/6304163.
10. Teufack, S.E.N., Tayo, G.N., Alidou, M.N., Yondo, J., Djiomene, A.F., Poné, J.W., & Mbida, F. M., 2017. Anthelminthic properties of Methylene chloride-methanol (1: 1) extracts of two ameroonians medicinal plants on Heligmosomoides bakeri (Nemato da: Heligmosomatidea). BMC Complement Altern Med. 17,400.https://doi.org/10.1186/s12906-017-1908-8.
11. OECD, T.N., 2001. 423: Acute Oral toxicity–Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals, Section, 4.
12. Patil, K.R., Patil, C.R., 2017. Anti-inflammatory activity of bartogenic acid containing fraction of fruits of Barringtonia racemosa Roxb. in acute and chronic animal models of inflammation. J Tradit Complement Med.7, 86-93. https://doi.org/10.1016/j.jtcme.2016.02.001.
13. Rai, U., Rawal, A., Singh, S., 2018.Evaluation of the anti-inflammatory effect of an anti-platelet agent crinumin on carrageenan-induced paw oedema and granuloma tissue formation in rats. Inflammopharmacology. 26, 769-778. https://doi.org/10.1007/s10787-017-0411-7.
14. Subash, A., Veeraraghavan, G., Sali, V.K, Bhardwaj, M., Vasanthi, H.R., 2016. Attenuation of inflammation by marine algae Turbinaria ornata in cotton pellet induced granuloma mediated by fucoidan like sulphated polysaccharide. Carbohydr Polym. 151, 1261-1268. https://doi.org /10.1016/j.carbpol.2016.06.077.
15. Ashok, P., Koti, B.C., Thippeswamy, A.H M., Tikare, V.P., Dabadi, P., Viswanathaswamy, A. H.M., 2010. Evaluation of antiinflammatory activity of Centratherum anthelminticum (L) Kuntze seed. Indian journal of pharmaceutical sciences, 72, 697-703.https://doi.org/10.4103/0250-474X.84577.
16. Fulgenzi, A., Dell'Antonio, G., Foglieni, C., Dal Cin, E., Ticozzi, P., Franzone, J. S., Ferrero, M.E., 2005. Inhibition of chemokine expression in rat inflamed paws by systemic use of the antihyperalgesic oxidized ATP. BMC Immunol.6, 18.https://doi.org/10.1186/1471-2172-6-18.
17. Chauhan P, Singh S, Gupta YK, Kumar U., 2018.Evaluation of toxicity studies and anti-inflammatory activity of Terminalia Bellerica in carrageenan-induced paw edema in experimental rats. J Nat Sc Biol Med. 9,169-174. https://doi.org/10.4103/jnsbm.JNSBM_159_17.
18. Ma, J., Guo, C., Pan, Y., Lin, D., Qiu, L., Wen, L., 2017. Antioxidant and anti-inflammatory activities of ethyl acetate extract of Gynura formosana (Kitam) leaves. Exp Ther Med. 14, 2303-2309. https://doi.org/10.3892/etm.2017.4757.
19. Sanmuga Priya E, Venkataraman S., 2007. Anti-inflammatory effect of Strychnos potatorum. seeds on acute and sub acute inflammation in experimental rat models. Pharmaceutical biology. 45, 435-439. https://doi.org/10.1080/13880200701388922.
20. Narendhirakannan, R.T., Subramanian, S., Kandaswamy, M., 2007. Evaluation of anti-inflammatory activity of Cleome gynandra L. leaf extract on acute and chronic inflammatory arthritis studied in rats. J Pharmacol Toxicol. 2, 44-53. https://doi.org/ 10.3923/jpt.2007.44.53