Research Article

# Anti–Inflammatory and Analgesic Activities of Methanol Extract of *Terminalia chebula* Fruits

**S. Kirubanandan[1], Bharathi Ravi[2] and S. Renganathan[1]**

[1]Centre for Biotechnology, A.C.Tech., Anna University, Chennai-600 025, Tamilnadu, India.
[2]Life Cell International Pvt Ltd, Chennai–600 127, Tamilnadu, India.

**ABSTRACT**
Phyto-pharmaceuticals have been recommended a number of pytochemicals as drugs from indigenous plant sources for the treatment of a variety of inflammation. The anti–inflammatory and analgesic activities of methanol extract of fruits of *Terminalia Chebula* were investigated in Wister albino rats and mice. The methanol extract of these fruits were found to encompass substantial anti–inflammatory effect in acute and sub-acute models and analgesic effect. Animal models of carrageenan–induced edema and cotton pellet induced granuloma in albino rats were performed. The Extract of 100 mg/kg shows significantly reduced paw edema. Analgesic activity of *Terminalia Chebula* fruits with 100 mg/kg is also shown by significant reduction of writhing. These report shows to support the use methanol extract of *Terminalia Chebula* fruits in relieving inflammation and pain.

**Keywords:** *Terminalia Chebula* Fruits, Anti–inflammatory activity, Analgesic activity.

## INTRODUCTION

Phyto-pharmaceuticals from medicinal plants are widely used in folk medicine of many countries to treat a variety of inflammatory conditions and in particular, dermal inflammations, broncho-inflammations and arthritis. A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred genera are used medicinally, mainly as herbal preparations in the indigenous systems of medicine in different countries and are sources of very potent and powerful drugs which have stood the test of time and modern chemistry has not been able to replace most of them. The World Health Organization reported that ~80% of the world's population rely chiefly on traditional medicine and a major part of the traditional therapies involve the use of herbal plant extracts or their active constituents/phyto extracts (Farnsworth, 1984: not cited in reference). The exposure of the presence of a variety of natural products such as polyphenols, alkaloids, flavonoids and other secondary metabolites in medicinal plants will provide a scientific justification for the popular use of them and serve as a guide which may help in the selection of the plants with anti-inflammatory and analgesic activity. Polyphenols present in plant have ability to inhibit lipoxygenase dependent peroxidation and to quench reactive oxygen species in various diseases such as auto-immune disease, inflammation and arthritis (Edwin Haslam, 1996: not cited in reference).

*Terminalia Chebula* fruits are regarded as a universal panacea in the ayurvedic medicine as well as in the traditional medicine. Their fruit has numerous pharmacological properties which were tabulated in Table 1(Gupta *et al*., 2012). Additionally, *T.Chebula* could protect the rat pheochromatoma cells in vitro from ischemic damage and its mechanism showed the inhibition of oxidation and inflammatory effects. (Gaire *et al*., 2013). Sukakul. T *et al*., 2013 confirmed that topical application of *T.Chebula* fruits extract on cronton oil-induced mouse ear dermatitis could help reducing inflammation. Md. Safkath Ibne Jami *et al.,* 2014., reported that ethanolic extract of *Terminalia chebula* fruits has significant analgesic and anti-inflammatory activities. In this work, the methanol extract of T.Chebula fruits is used to evaluate its anti-inflammatory potential and analgesic activity using *in vivo* studies.

## MATERIALS AND METHODS

### Plant Material and Extraction

The fruits of the *Terminalia chebula* were collected from CLRI institute campus (Central Leather Research Institute, Chennai, India). The fruits were separated and shade dried. The dry fruits were grounded into powder form using the grinder. Extraction was performed by using Soxhlet apparatus with Methanol. The resultant extraction was evaporated to dryness under reduced pressure in Rotary vacuum evaporator 40-45$^{o}$C. The concentration extract was aliquoted in amber-colored bottles and kept in desiccators for further use.

### CHEMICALS AND DRUGS

Carrageenan (Sigma, USA) was used for induction of inflammation. Indomethacin (Recon, Bangalore, India) were used as the standard drug. All other reagents were used analytical grade.

### Animals

Inbred colony of adult male Wistar albino rats (170–200 g) and male Swiss albino mice (20-25 g) were used for the pharmacological activities. They were kept in polypropylene cages at 25 ± 2° C, with relative humidity of 45-55% under 12h:12h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed (Poultry Research Station, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.) and water *ad libitum.* The test extracts and the standard drugs were administered in the form of a suspension in water using 1% Tween 80% as suspending agent. All the pharmacological experimental protocols were approved by the Institutional animal ethics committee.

### Phytochemical screening of methanol extract of *T.Chebula* Fruits:

Preliminary phytochemical screening of the plant extract using the standard methods of Trease and Evans (1989) gave positive tests for polyphenols and tannins.

### Carrageenan–induced edema test

Paw edema was induced by injecting 0.1ml of carrageenan dissolved in saline containing 1% Tween 80 into the sub plantar tissues of the left hind paw of each rat (Winter *et al.,* 1962). The methanol extract of T.Chebula Fruits (100 & 200 mg/kg,) was administered orally 30 min prior to carrageenan injection. The paw volume was measured at 60, 120, 180, 240 minutes by the mercury displacement method using a plethysmometer. The percentage inhibition of paw volume in drug treated group was compared with the control group. Indomethacin (10 mg / kg of b.w., p.o.) was used as a reference standard.

### Cotton pellet induced granuloma:

Male Wister albino rats (170 -200 g) were divided into 4 groups of 6 animals in each group. Cotton pellets weighing 30±1mg were autoclaved and implanted subcutaneously into both sides of the groin region of each rat (D'Arcy *et al*., 1960). Group I served as control and received the vehicle. The methanol extract of *T.Chebula* fruits at concentrations of 100 and 200 mg/kg was administered orally for Group II and III animals for 7 days. Group IV animals received indomethacin at a dose of 10 mg/kg orally for same period. On the 8th day the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60$^{o}$C, weighed and compared with control.

### Acetic acid-induced writhing test

One hour after receiving oral (p.o.) administration of the plant extract, reference substance or solvent to groups of 6 mice, each mouse was given intraperitoneally 0.6% aqueous solution of acetic acid (2 ml/kg body weight)( Franzotti *et al.,* 2002). Immediately after the algic compound injection, each animal was placed in a transparent observation cage and the number of writhes per mouse was counted for 30 minutes. The writhing activity consists of a contraction of the abdominal muscles together with a stretching of the hind limbs.

### Statistical analysis

Values were expressed as mean ± SEM. Statistical significance was determined by ANOVA, followed by Student's t–test; values with $p<0.05$ and $p<0.01$ were considered as statistically significant.

## RESULTS

In the present phyto-chemical investigation, the methanol extract of *T.Chebula* fruits contains tannins, polyphenols and flavanoids (Table 1). It has reported that the major constituents of *T.Chebula* Fruits are 30 % Tannins and Poly Phenols.

### Anti-inflammatory activity

The anti-inflammatory activity of methanol extract of *T.Chebula* (MET) was investigated at the dose of 100 and 200 mg/kg body weight against acute paw edema induced by

carrageenan, formalin and cotton pellet induced granuloma. In the carrageenan-induced rat paw edema model, the MET produced significant ($p<0.01$) anti-inflammatory activity and the results were comparable to that of indomethacin as a standard anti–inflammatory drug. The MET at the doses of 100 and 200 mg/kg of bw showed inhibition of 36.3% and 54.5% at the third hour after carrageenan injection whereas Indomehacin shows 63% inhibition. The methanol extract of *T.Chebula* fruits showed significant inhibitory effect on the edema formation (Fig. 1).

In cotton pellets induced granuloma model, the effects of MET and indomethacin on the proliferative phase of inflammation are summarized. It was seen that MET (Methanol Extract of *T.Chebula*) was responsible for anti-inflammatory effect, which would be calculated depending on the moist and dry weight of cotton pellets. According to these results, the anti-proliferate effects of MET at the dose of 200 mg/kg and indomethacin were investigated as 43.61% and 55.29% ($p<0.05$), on wet basis respectively. After they were dried, the anti-proliferative effects were calculated on the basis of dry weight pellets, and the inhibitions of inflammation by MET 200 mg/kg and indomethacin were investigated as 51.8 and 60.45 % ($p<0.05$), respectively (Table 1).

**Analgesic activity**

In acetic acid induced writhing in mice model, analgesic effects induced by different doses of MET on the writhing test in mice are shown in Table 3. The MET at the dose of 100 and 200 mg/kg bw and indomethacin of 10mg/kg bw exhibited significant ($p< 0.01$) inhibition of the writhes at the rate of 31.5, 51.8 and 64.0%, respectively. In synergistic addition, MET + indomethacin at the different doses also potentiated (72.3 and 78.0 %) the indomethacin-treated analgesia (Table 2).

**Table 1: Effect of methanol extract of *T.Chebula* on cotton pellet induced granuloma in rats.[#] Values are mean ± SEM (n=6),* p< 0.05, ** p< 0.01 compared with control**

| S. No | Treatment | Dose (mg/kg) | Weight of cotton pellet[#] (mg) (Moist) | Inhibition (%) | Weight of cotton pellet[#] (mg) (Dried) | Inhibition (%) |
|---|---|---|---|---|---|---|
| 1 | Control | 2ml/kg, p.o 1% Tween80 | 187.33±4.06 | | 43.16±1.64 | |
| 2 | Extract | 100 | 125.50±2.98 | 35.07 | 29.50±1.50 | 34.96 |
| 3 | Extract | 200 | 108.31±4.74* | 43.61 | 22.40±1.38* | 51.18 |
| 4 | Indomethacin | 10 | 86.76±2.45** | 55.29 | 18.50±1.54* * | 60.45 |

**Table 2: Effect of methanol extract of *T.Chebula* Extract on acetic acid induced writhing test in mice**

| S. No | Treatment | Dose (mg/kg) | No. of writhing[#] | Inhibition (%) |
|---|---|---|---|---|
| 1 | Control | 2ml/kg, p.o 1% Tween80 | 39.5±1.6 | - |
| 2 | Indomethacin | 10 | 16.5±1.0** | 64 |
| 3 | Extract | 100 | 25.6±1.4* | 31.75 |
| 4 | Extract | 200 | 16.8±1.3** | 56.28 |
| 5 | Extract+Indomethacin | 100 +10 | 10.6±1.3** | 72.38 |
| 6 | Extract+Indomethacin | 200+10 | 8.4±1.2** | 78 |

[#]Values are mean ± SEM (n=6)

*p< 0.05,** p< 0.01 compared with control.

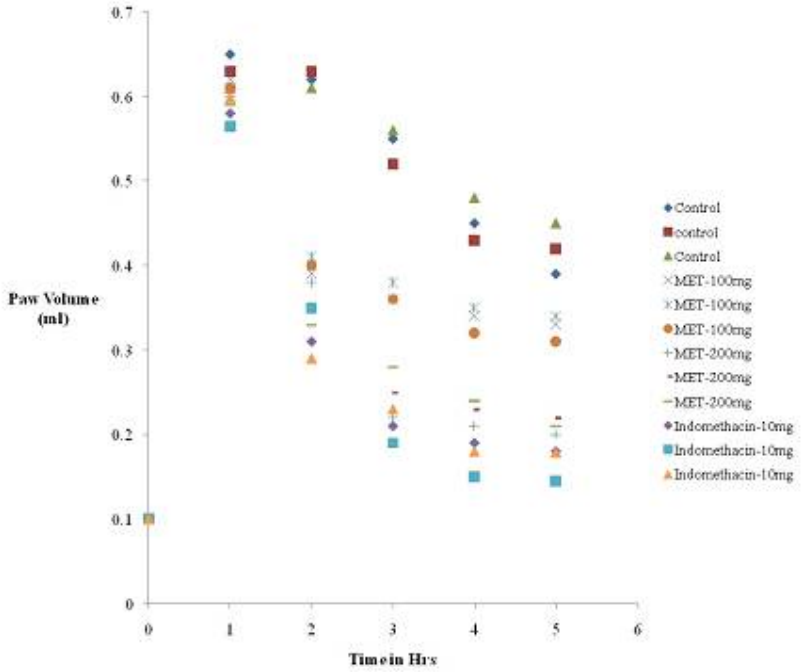


**Fig. 1: Effect of methanol extract of *T.Chebula* Fruits (MET) on carrageenan induced Paw edema test in rats**

## DISCUSSION

In Indian system of medicine, a variety of medicinal herbs are claimed to provide relief of pain and inflammation. The claimed therapeutic reputation has to be verified in a scientific approach. The methanol extract of *T.Chebula* fruits possess significant anti-inflammatory effect in the acute and sub-acute inflammation in rats. Carrageenan induced acute inflammation model is one of the most suitable test procedures to screen anti–inflammatory agents from a variety of plant extracts. The development of carrageenan–induced edema is biphasic, the first phase is attributed to the release of histamine, 5-HT and kinins, while second phase is related to the release of prostaglandins (Larsen *et al.,* 1983). The methanol extract of *T.Chebula* fruits showed a dose dependent anti–edematogenic effects on paw edema. The results suggest that the main mechanism of action may involve the inhibition of prostaglandin biosynthesis pathway and also influence other mediators of inflammation. Inflammation is a complex chronic process. Cotton pellet induced granuloma is a typical feature of an established chronic inflammatory reaction and can serve as a sub-chronic and chronic inflammatory test model for investigation of anti-arthritic substances. This model has been employed to assess the transudative and proliferative components of chronic inflammation. The fluid adsorbed by the pellet greatly influences the wet weight of the granuloma whereas the dry weight correlates well with the amount of granulomatous tissue formed. Administration of methanol extract of *T.Chebula* fruits (100 and 200 mg/kg bw) and indomethacin (10 mg/kg bw) appear to be effective in inhibiting the wet weight of cotton pellet. On the other hand, the methanol extract of *T.Chebula* fruits effect on dry weight of cotton pellet was almost near to that of indomethacin.

The acetic acid induced abdominal writhing which is the visceral pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis, and plays a role in the nociceptive mechanisms (Franzotti *et al.,* 2002). Results of the present investigation show that all the doses of the methanol extract of *T.Chebula* has potential of a significant analgesic effect and this effect may be due to inhibition of the synthesis of the arachidonic acid metabolite.

The mechanism of anti-inflammatory action of methanol extract of T.Chebula fruits may be due to its anti-oxidant activity. Reactive oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis, and aging process (Guyton *et al.,* 1997). Inflammation is a complex

process and ROS plays an important role in the pathogenesis of inflammatory diseases (Conner and Grisham, 1996). Thus, antioxidants that scavenge ROS are expected to improve these disorders. *T.Chebula* fruits have anti-oxidant activity to quench free radicals formed in tissues, and this activity may decrease inflammation (Naik *et al,* 2005). *T. chebula* fruit has high phenolic content and strong inhibition of *in vitro* lipid peroxidation (Naik *et al.,* 2005). Md. Safkath Ibne Jami *et al.,*2014., reported that ethanolic extract of *Terminalia chebula* fruits has significant analgesic and anti-inflammatory activities at a concentration of 250 mg of extract /Kg of body weight which is confirmed effective concentration of pharmacological activities. In our investigation, 100mg of *T.Chebula* extract/ kg of body are an optimized concentration of producing anti-inflammatory activity and analgesic activity in mice and rats. The isolated bio active molecules from *T.Chebula* Extract will be studied on inflammatory pathway such as lipooxygenase and cyclooxygenase pathway.

## CONCLUSION

This study supports the contention that traditional medicines remain a valuable resource in the potential discovery of Phyto pharmaceuticals and lead molecules from medicinal plants. A remarkable anti inflammatory and analgesic activity exhibited by methanolic extract of '*T.Chebula* Fruits' provides a scientific validation for the popular use of this herbal drug and further work will be investigated on isolation and identification of bio active compounds which could influence on the inflammation pathway.

## ACKNOWLEDGMENTS

The authors are grateful to Department of Pharmacology, C.L.Baid Mehta College of Pharmacy, Chennai, India, for the constant support and encouragement throughout this In vivo study.

## REFERENCES

1. Ahmad I, Mehmood Z and Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethno-pharmacology. 1998;62:183-193.
2. Brooks PM and Day RO. Non-steroidal anti-inflammatory drugs: differences and similarities. N Engl J Med. 1991;3:241-246.
3. Conner EM and Grisham MB. Inflammation, free radicals and antioxidants, Nutrition. 1996; 12:274-7.
4. Guyton KZ, Gorospe M and Holbrook NJ. Oxidative stress and the molecular biology of antioxidant defenses. Scandalios, J. G. Ed. Cold Spring Harbor Laboratory Press, New York, 1997;247-272.
5. Halliwell B and Gutteridge JMC. Free radicals in biology and medicine, Oxford University Press, Oxford. 1999.
6. Larsen GL and Henson PM. Mediators of Inflammation, Annu Rev Immunol. 1983;1:335.
7. Safkath Ibne Jami MD, Zakia Sultana, Ershad Ali MD, Marium Begum MST and Mominul Haque MD. Evaluation of Analgesic and Anti-Inflammatory Activities on Ethanolic Extract of Terminalia chebula Fruits in Experimental Animal Models. American Journal of Plant Sciences. 2014;5:63-69.
8. Nadkarni AK. Indian Materia Medica, 3rd Ed. Popular Press Ltd. Mumbai, India. 1976.
9. Prakash Chandra Gupta. Biological and Pharmacological properties of Terminalia Chebula Retz. - An Overview. International Journal of Pharmacy and Pharmaceutical Sciences. 4, 3, 62-68.
10. Sukakul T, Kettawan A, Chompoopong S and Rungruang T. Topical application of Terminalia chebula extract helps croton oil-induced dermatitis in mice. International Food Research Journal. 2013; 20(5):2269-2272.
11. Tokura K and Kagawa S. Anticancer agents containing chebulanin from Terminalia chebula.' Jpn. Kokai Tokkyo Koho JP. 1995;07:138- 165.
12. Trease GE and Evans WC. (1989).Text book of pharamacognosy, 13rd Ed. Oxford University Press. Oxford.
13. Vane J and Booting R. Inflammation and the mechanism of action of anti-infalmmatroy drugs . FASEB J. 1987;1: 89.
14. Winter CA, Risley EA and Nuss GW. Carrageenan induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Bio Med. 1962;111:544-547.