Evaluation of *in-vitro* anti-inflammatory activity of chebulinic acid from *Terminalia chebula* Linn. against the denaturation of protein

*Sima Singh and Uma Ranjan Lal**

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, INDIA- 835215

Email:uma@bitmesra.ac.in

Abstract: The present study highlights the importance of *Terminalia chebula* Linn, a plant used in traditional system of medicine for various disorders. This study was aimed at the evaluation of the anti-inflammatory activity of marker compound (chebulinic acid) from the pericarp of fruits of *Terminalia chebula*. There have been a number of reports in the literature, where the hydrolytic products of chebulinic acid i.e. gallic acid, ellagic acid and galloyl derivatives have shown marked anti-inflammatory properties, however the anti-inflammatory properties of chebulinic acid have not been determined. As denaturation of protein is one of the major reasons, which is responsible for inflammation, the activity was determined in terms of its ability to inhibit denaturation of proteins. Chebulinic acid was incubated at different concentrations ranging from 0.0 - 100.0 µg/ml with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Chebulinic acid efficiently reduced the denaturation of protein in terms of percentage inhibition (IC_{50} - 43.92 µg/ml). The percentage inhibition was comparable with that of standard (diclofenac sodium) having IC_{50} value 47.04 µg/ml. Our study is the first report which focuses on anti-inflammatory response of chebulinic acid against denaturation of proteins. Since, the results are comparable with diclofenac sodium. The plants as well as compound can be taken for further *in-vivo* studies.

Keywords: Chebulinic acid, denaturation of protein, anti-inflammatory activity, *Terminalia chebula*

Introduction

Terminalia chebula (Combrataceae) is commonly known as *Haritaki* in India. It is found throughout India and Southeast Asia in deciduous forest and areas of light rainfall **[1, 2].** *Terminalia chebula* (pericarp) is indicated for various disorders in Ayurveda including cancer, paralysis, cardiovascular disorders ulcers, leprosy, antidiabetic and wound healing activities etc. It is used extensively in the preparations of many ayurvedic formulations. Chebulinic acid, chebulagic acid, chebulic acid, gallic acid, ethyl gallate and galloyl derivatives are the major phytoconstituents present in the pericarp of *Terminalia chebula* fruits . Chebulinic acid is the main marker constituent of the fruits and is found upto 6-7% in the fruit. Its hydrolytic products are gallic acid, ellagic acid, mono and di-galloyl derivatives and chebulic acid (figure 1). Mention of hydrolytic product is of importance as many of its principle preparations (ayurvedic) involve preparation of decoction of the fruit; and it is likely that during making of the formulations chebulinic acid is hydrolysed and they in turn may be biologically active **[2, 3]**. *Terminalia chebula* and its preparations are mainly indicated for gastro-intestinal disorders and most dreaded gastro-intestinal disorders (gastric ulcers, Crohn's disease, ulcerative colitis) arise due to inflammation only. So, the present work is based on determination of *in vitro* anti-inflammatory activity of chebulinic acid (denaturation of protein). In present discussion, the *in-vitro* anti-inflammatory activity of chebulinic acid against the denaturation of protein is discussed.

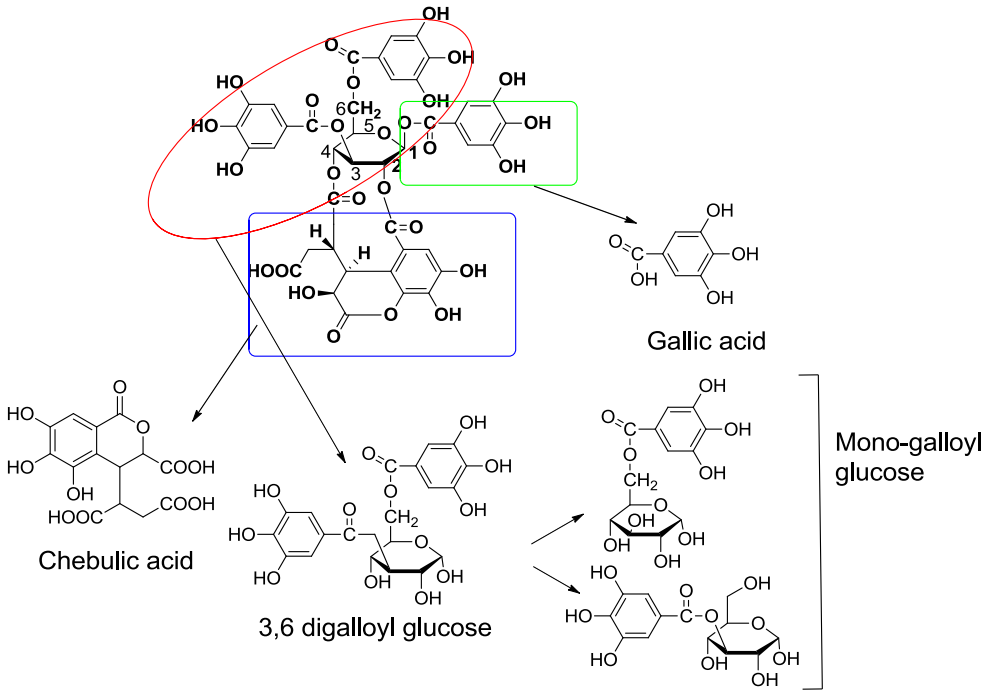

Figure 1: Chebulinic acid and its hydrolytic products.

EXPERIMENTAL SECTION

Isolation and characterization of marker constituents

Dried powder of pericarp (200 g) was extracted with acetone (2 L) in soxhlet extractor (not more than eight syphoning). The extract was filtered and concentrated to $1/7^{th}$ of the volume (350 mL). The insoluble were separated and the settle down the white crystallized substance. The water was decanted and crystalline powder material was further recrystallised with acetone to obtain rhombic crystals of chebulinic acid (700 mg).

Chebulinic acid **:** MALDI/TOF (DHB): *m/z* $[M+23]^+$ 979 , ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm):, 7.26 (s, 1H), 6.90, 6.87, 6.77 (s, 2H), 6.20 (1H, d, *J* = 2.8), 5.93 (1H, d =3.6), 5.11(1H, d, *J* = 8.0), 4.82 (1H, d, *J* =3.6) , 4.67 (1H, d, *J*=8.0), 4.49 (m), 4.27(1H, m), 4.31(1H, m), 3.61 (1H, m), 1.81 (1H, m),1.93 (1H, m), ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 173.2, 172.5, 169.5, 165.6, 164.5, 164.1, 162.0, 146.1, 145.9, 145.8, 145.7, 140.5, 139.8, 139.9, 118.9, 117.7, 116.3, 115.8, 115.0, 109.2, 108.8, 91.9, 76.1, 72.3, 68.6, 65.2, 64.3, 62.2.

Preparation of test solution

Test solution of chebulinic acid of varying concentrations ranging from (0.0 µg/ml-100.0 µg/ml) was prepared in phosphate buffer of pH 7.4.

Preparation of standard solution

Standard solution of declofenac sodium of varying concentration (0.0 µg/ml-100.0 µg/ml) was prepared in phosphate buffer of pH 7.4.

Evaluation of in vitro anti-inflammatory activity

In-vitro anti-inflammatory activity of chebulinic acid against denaturation of protein was carried out as per method described by Mizushima and Kobayashi **[4]**. The 5 ml of reaction mixture consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffered saline (PBS, pH 7.4) and 2 ml of different concentrations of chebulinic acid so as to obtain the final concentrations. Equal volume of triple distilled water served as control. After that the mixtures were incubated at (37±2) °C in a BOD incubator (Navyug, India Ltd) for 30 minutes and heated at 70°C for 15 minutes. After cooling, the absorbance was measured at 280 nm by UV spectrophotometer (Shimadzu 1800, Japan) by using vehicle as blank and the viscosity was determined by using Ostwald Viscometer. Same procedure was followed for the standard

solutions. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = (Vt / Vc - 1) \times 100$$

Where, *Vt* = absorbance of test sample, *Vc* = absorbance of control. The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS

The results obtained are summarized in **Table 1**. The present findings suggest that inhibition of denaturation of protein by chebulinic acid is concentration dependent in the range of (0.00 µg/ml-100 µg/ml). The concentrations of Diclofenac sodium, used in the experiments were same as that of chebulinic acid.

Table (1): Effect of Diclofenac Sodium and Chebulinic Acid on protein denaturation.

S. No.	Concentration (µg/ml)	Effect of diclofenac sodium on protein denaturation		Effect of chebulinic acid on protein denaturation	
		% inhibition	Viscosity (cp)	% inhibition	Viscosity (cp)
1	0.00	0.00	0.00	0.00	0.00
2	10.0	8.00	0.54	7.00	0.49
3	20.0	18.02	0.60	14.58	0.57
4	30.0	26.90	0.66	24.65	0.62
5	40.0	39.04	0.72	36.42	0.68
6	50.0	47.04	0.76	43.92	0.72
7	60.0	52.71	0.79	47.61	0.76
8	70.0	57.04	0.84	52.04	0.82
9	80.0	62.52	0.88	58.04	0.85
10	90.0	68.86	0.92	63.04	0.89
11	100.0	72.04	0.98	67.04	0.94

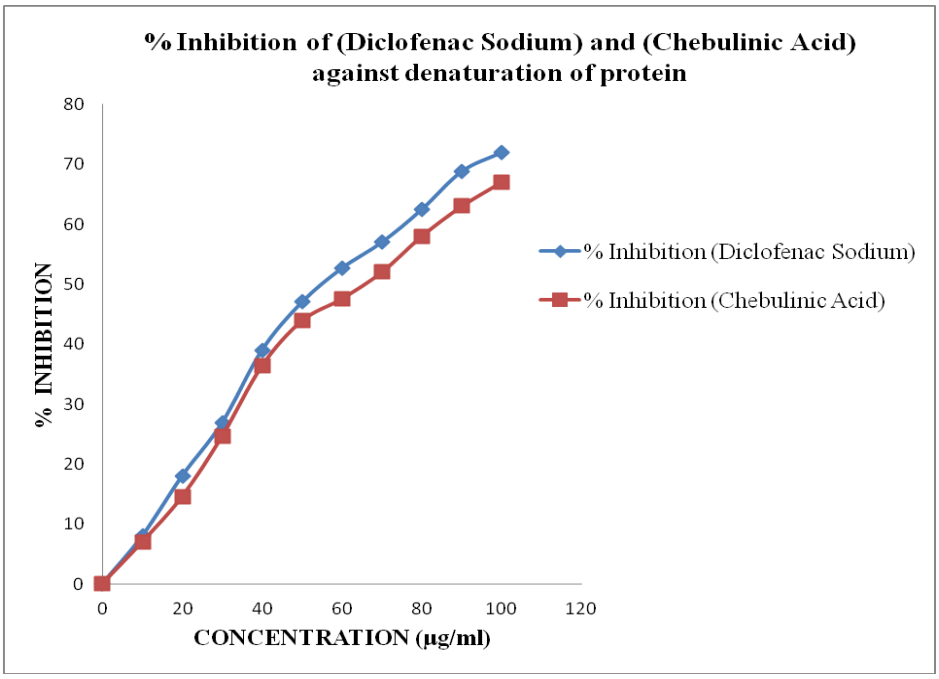

Figure 2: Percentage inhibition of Diclofenac Sodium and Chebulinic acid against denaturation of protein

From the results it is evident that chebulinic acid efficiently reduces the denaturation of protein in terms of percentage inhibition (IC_{50} - 43.92 µg/ml). The percentage inhibition was comparable with that of standard (diclofenac sodium) having IC_{50} value 47.04 µg/ml. Our study is the first report which focuses on anti-inflammatory response of chebulinic acid against denaturation of proteins. Since, the results are comparable with diclofenac sodium. The plants as well as compound can be taken for further *in-vivo* studies.

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