International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Anti-inflammatory potential of green synthesized silver nanoparticles of triphala extract

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International Journal of Science and Research Archive, 2022, 07(02), 168–172

Publication history: Received on 03 October 2022; revised on 14 November 2022; accepted on 17 November 2022

Article DOI: https://doi.org/10.30574/ijsra.2022.7.2.0252

Abstract

The present study was to investigate the *in vitro* anti-inflammatory activity of the hydroalcoholic extract of triphala and its green synthesized silver nanoparticles using the human red blood cells membrane stabilization method and protein denaturation method and the results were compared with standard diclofenac sodium. The presence of flavonoids, phenolics, alkaloids, carbohydrates, and glycosides were found in phytochemical screening. The plant extract and the silver nanoparticles showed positive anti-inflammatory activity through HRBC membrane stabilization and inhibition of protein denaturation in a concentration-dependent manner. The study was concluded that the hydroalcoholic extract and SNPs of triphala exhibited potent anti-inflammatory agent due to the presence of important chemical constituents such as polyphenols and flavonoids and this anti-inflammatory properties help in the prevention of many chronic disorders.

Keywords: Anti-inflammatory; HRBC membrane stabilization; Protein denaturation; Diclofenac sodium

1. Introduction

Inflammation occurs in response to infectious microorganisms such as bacteria, viruses or fungi invade, reside in tissues or circulate in the blood and also due to cell death, injury to tissues, cancer, ischemia and degeneration in which both innate and adaptive immune responses are involved [1]. When damage or injury occurs, inflammation is started at the site by migrating immune cells, releasing inflammatory mediators, recruiting cells of inflammation and releasing free radicals and cytokines to settle the infection and repair the tissue injured. Thus, the normal inflammation which is for host defense is rapid and beneficial but the chronic inflammation produces many chronic disorders such as cancer, metabolic disorders, cardiovascular disorders and neurological disorders and it can be prevented by the abolition of the chronic inflammation [2,3].

Triphala is a very common and important ayurvedic preparation composed of *Terminalia chebula*, *Terminalia belerica*, and *Emblica officinalis* and is termed as tridoshic rasayana to have longlife and rejuvenating effects on human life. Triphala is used in gastric, intestinal disorders, cardiovascular disorders, respiratory system disorders, liver dysfunction, anemia, inflammation, opthalmic problems, and possesses various antimicrobial activities such as antibacterial, antifungal, antimalarial, and antiviral activities and also having antineoplastic, chemoprotective activities, antiaging properties and improving mental health [4,5,6]. *T. chebula* fruits containing constituents such as tannins, flavonoids, saponins, anthraquinones, starch, fructose, aminoacids, fatty acids and succinic acid and *Terminalia bellerica* fruits contain proteins, oils and linoleic acid as main constituents. E. officinalis are rich in ascorbic acid,

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ellagitannins such as emblicanin A and emblicanin B, phyllemblin, phyllanemblinin A, flavonoids, gallic acid, ellagic acid and kaempferol and the various health benefits of triphala might be due to the presence of these important chemical constituents [7].

2. Material and methods

2.1. Collection of plant material

The triphala components such as *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis* were collected from Kannur district in Kerala, India in October 2020 and were authenticated by Dr. P. Sreeja Msc, PhD, Assistant Professor and Senior Research Officer, P G Department of Botany and Research Centre, Sir Syed College, Thaliparamba, Kannur, Kerala. A voucher specimen No-99366 was deposited in the P G Department of Botany and Research Centre Herbarium, Sir Syed College, Thaliparamba, Kannur, Kerala for future reference. Dried fruits were ground to get coarse powder, and then passed through sieve no 24, mixed well, airtight container used for storing, and then extracted.

2.2. Preparation of hydro alcoholic extract of Triphala (HAET)

HAET was prepared by percolation method (about 48 h). The triphala powder (200 g) was extracted with hydroalcoholic solution (96%, 500 mL) using a 2 L percolator. The extract was concentrated in rotary evaporator to 50 mL, and then freeze- dried [8].

2.3. Preliminary phytochemical screening

Triphala was tested using standard methods and confirmed the presence or absence of phytoconstituents such as flavonoids, polyphenols, alkaloids, carbohydrates, proteins, tannins, glycosides, and steroids.

2.4. Green synthesis of Triphala-Silver Nanoparticles (TSNPs)

10.0 g of dried extract was suspended in 100 ml of 1mM silver nitrate (AgNO3) solution in 1:10 ratio (w/v). The mixture was centrifuged at 6000 rpm for 30 minutes, double filtered and the supernatant used for further experiments. The nanoparticles were further characterized by UV-Visible spectroscopy and Transmission electron microscopy (TEM) [9].

2.5. Determination of anti-inflammatory activity of Triphala extracts and TSNPs

2.5.1. The human red blood cell (HRBC) membrane stabilization method

The blood was mixed with an equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% sodium chloride) and centrifuged at 3000 rpm. The packed cells were washed with saline and a 10% suspension was made. Various concentrations of extracts are prepared by using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hypo saline, and 0.5% of HRBC suspension were added. It was incubated at 37 ℃ for 30 minutes and centrifuged at 3000 rpm for 20 minutes. The hemoglobin content of the supernatant solution was estimated spectrometrically at 560 nm. Diclofenac sodium was used as the standard and control prepared by omitting the extracts [10,11]. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula,

$$\% \text{ protection} = \frac{opticaldensityofdrugtreatedsample}{opticaldensityofcontrol} \times 100$$

$$\%\text{inhibition} = 1 - \frac{opticaldensityofdrugtreatedsample}{opticaldensityofcontrol} \times 100$$

2.5.2. Protein denaturation method

Dilutions of extracts and standard drug (diclofenac sodium) were prepared in concentrations of 12.5-200 µg/ml. To 0.2 ml egg albumin, 2.8 ml phosphate buffered saline (PBS) of pH 6.4 and 2 ml of varying concentrations of extracts or standard were mixed. A similar volume of distilled water was taken as control. The mixtures were incubated at 37 ℃ in the incubator for 15 minutes and then heated at 70 ℃ for 5 minutes. After cooling, absorbance was measured at 660 nm by using vehicle as blank [12,13]. The inhibition of protein denaturation is calculated by the formula;

$$\% \text{ Inhibition} = 1 - \frac{absorbanceoftest}{absorbanceofcontrol} \times 100$$

3. Results and discussion

3.1. Preliminary phytochemical screening

The presence of flavonoids, polyphenols, alkaloids, carbohydrates, proteins, glycosides, and steroids was found in the preliminary phytochemical screening.

3.2. HRBC membrane stabilization method:

The anti-inflammatory potential of the HAET and TSNPs were evaluated using the HRBC membrane stabilization method. The extract exhibited anti-inflammatory activity in a concentration-dependent manner. The IC_{50} values of the standard diclofenac sodium was found to be 56.27 µg/ml, the hydroalcoholic extract showed 96.81 µg/ml, and the silver nanoparticles were found to be 68.04 µg/ml. At a concentration of 640 µg/ml the hydroalcoholic extract showed maximum stabilization of 80.35% while the standard diclofenac sodium exhibited maximum stabilization of 91.24% and silver nanoparticles showed 89.06%. (Table 1 and Figure 1).

Table 1 *In vitro* anti-inflammatory activity of silver nanoparticles using HRBC membrane stabilization method

Groups	Concentration (µg/ml)	% Stabilization	IC50 (µg/ml)
DFS	40	41.84	56.27
	80	62.44	
	160	70.91	
	320	79.57	
	640	91.24	
HAET	40	36.83	96.81
	80	48.32	
	160	58.33	
	320	69.58	
	640	80.35	
TSNPs	40	39.04	68.04
	80	57.74	
	160	66.33	
	320	77.24	
	640	89.06	

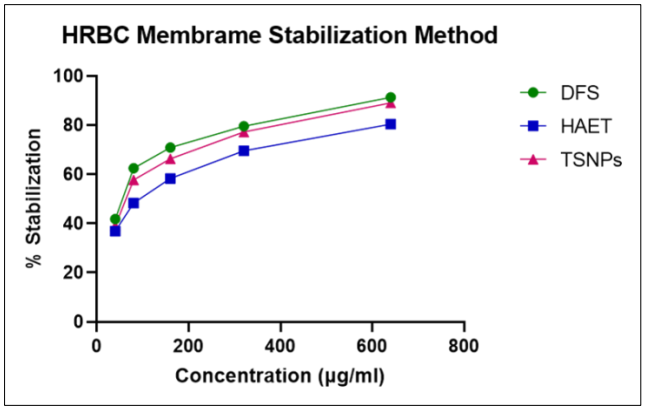

Figure 1 *In vitro* anti-inflammatory activity of silver nanoparticles using HRBC membrane stabilization method

3.3. Protein denaturation method

The hydroalcoholic extract of triphala and its silver nanoparticles was effective in protein denaturation inhibition. The standard diclofenac sodium showed IC50 value 27.65 µg/ml and hydroalcoholic extract and silver nanoparticle showed IC50 values 54.78 µg/ml and 35.81 µg/ml respectively. The percentage inhibition of HAET and TSNPs at concentration 640 µg/ml was found to be 82.08% and 89.48% respectively and diclofenac sodium showed 92.35% inhibition at the same concentration. (Table 2 and Figure 2).

Table 2 *In vitro* anti-inflammatory activity of silver nanoparticles by protein denaturation method

Groups	Concentration (µg/ml)	% Inhibition	IC50 (µg/ml)
DFS	20	43.01	27.65
	40	59.28	
	80	69.04	
	160	77.04	
	320	85.77	
	640	92.35	
HAET	20	37.65	54.78
	40	48.32	
	80	57.54	
	160	66.61	
	320	79.03	
	640	82.08	
TSNPs	20	40.34	35.81
	40	56.19	
	80	64.68	
	160	73.62	
	320	84.07	
	640	89.48	

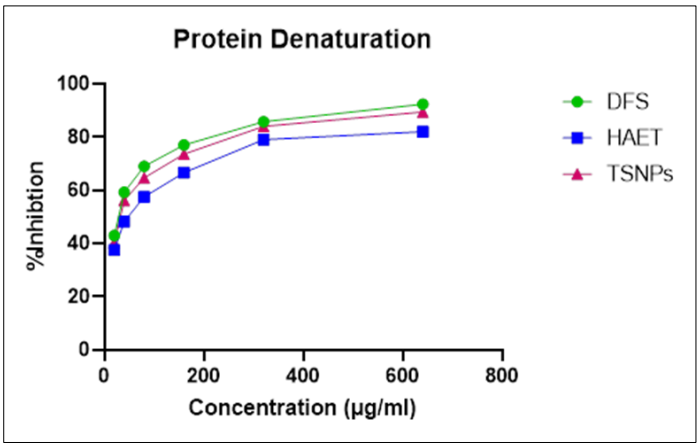

Figure 2 *In vitro* anti-inflammatory activity of silver nanoparticles by protein denaturation method

4. Conclusion

The hydroalcoholic extract of triphala and its silver nanoparticles showed potential anti-inflammatory activity compared with diclofenac sodium by the methods HRBC membrane stabilization and protein denaturation. The extract was effective in its nanoparticle form and these activities are due to the presence of important chemical constituents present in it. To explore more anti-inflammatory benefits the *in vivo* studies and isolation of constituents also should be performed and triphala will be a proper drug with more efficacy and lesser side effects.

Compliance with ethical standards

Acknowledgments

We would like to acknowledge Shri Jagdishprasad Jhabramal Tibrewala University.

Disclosure of conflict of interest

All authors declare there is no conflict of interest in this paper.

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