

Note

An *in vitro* evaluation of *Tribulus terrestris* L. fruit extract for exploring therapeutic potential against certain gut ailments

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The enteric pathogens and oxidative stress are known to generate intestinal inflammation, chronic gut ailments and oncogenesis. Modulation of the gut microbial peak populations through herbal agents, offers a promising therapeutic modality. *Tribulus terrestris* Linn. (Tt), a widely documented medicinal herb in Ayurveda, was investigated for antioxidant, anti-inflammatory and antimicrobial activities *in vitro*. Fruit extract of Tt and quercetin, evaluated for free radical scavenging by DPPH method, revealed IC₅₀ values as 98.83 and 24.77 µg/mL, respectively. Anti-inflammatory attributes of Tt fruit extract and indomethacin, a known anti-inflammatory drug, rendered IC₅₀ values as 10.8 and 12.9 µg/mL against protein denaturation. MTT assay on HCT-15 cells revealed a decrease in viability from 78 to 22% against 30 and 70 µg/mL of Tt fruit extract, respectively. Zone of inhibition against *E. coli* increased from 0.19 to 9.82 cm² at 200 and 1000 µg/mL of Tt, respectively. The fruit extract of Tt enhanced the growth of probiotic *Lactobacillus rhamnosus* (L.rh) by 19, 44 and 50 % over the control at 100, 150 and 200 µg/mL, respectively. This study indicated the potential of *Tribulus terrestris* fruit extract against inflammatory, oxidative and microbe generated pathogenic ailments in the digestive system.

Keywords: Anticancer, Antioxidant, Anti-inflammatory, Antimicrobial, Ayurveda, Cytotoxicity, Devil's horn, Gokshura, Gut microbiota, Inflammation, *Lactobacillus rhamnosus*, Probiotics, Puncture vine

In the digestive system, the gut microbiota, the dietary components, host gut tissue and secretions interact intensively and produce the metabolome. The variability of these factors in the gastrointestinal (GI) system of an individual at various stages generates a dynamic luminal environment and influences the peak populations of microbes which influence oxidative species, inflammatory molecules and pathologies in some cases. Thus, the changes in the luminal environment of the gut of the host initiate chronic and

acute discomforts and certain ailments, such as bloating or gas, distention, constipation, cramping and diarrhea. The chronic afflictions later may lead to the development of functional bowel syndrome such as IBS which is associated with impaired quality of life and severe morbidity¹. The metabolome contains the nutrients, building molecules including energy molecules which are transported to all the cellular entities, tissues and organs. Sometimes metabolome may also carry some undesirable components which may initiate various chronic pathologies too adding a substantial socioeconomic burden^{2,3}.

Available reports in biomedicine extensively enumerate the influence of gut microbial populations on human health^{4,5}. Substantial data on dynamics of microbial peak populations suggest that chronic intestinal inflammation is associated with enhanced production of reactive oxygen species (ROS). In ulcerative colitis, increased ROS levels and decreased endogenous anti-oxidants available in the mucosa have been associated with the inflamed mucosa^{6,7}. The increased oxidative stress has been reported to enhance protein denaturation and disrupt the integrity of gut barrier^{8,9}. The chronic inflammation and oxidative stress have also been associated with carcinogenesis¹⁰.

Various drugs available in modern medicine system include anti-inflammatory agents (steroidal and non-steroidal), immunosuppressants and biological drugs which often yield relief for shorter spans and also manifest sub-toxic to toxic symptoms later¹¹. In this context, natural products are not only acceptable to our body but also have either little or no adverse effects. Ayurveda, the traditional medicine system in India, has immensely relied on attributes of metabolome. Modulation of the gut microbial peak populations through herbs can yield antioxidative, anti-inflammatory and antimicrobial activity which can be exploited as a therapeutic modality. The bioactive molecules from the metabolome are transported across the membrane barrier into the cellular milieu and membrane receptors play an important role in the process. This permits the differential absorption of various bioactive molecules by different cells and tissues in a living system and can be translated for therapeutic manifestations.

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Tribulus terrestris (Tt), commonly known as puncture vine or devil's horn, is native to warm temperate and tropical regions of southern Europe, southern and western Asia, throughout Africa and Australia. *T. terrestris* is a known therapeutic agent by itself and as a primary or secondary component of many combinational formulations used in Ayurvedic system of medicine also. Its fruits have been used for treatment of eye ailments, abdominal distention, edema, hypertension, sexual dysfunction and urogenital disorders^{12,13}. *T. terrestris* has been reported to contain several alkaloids, tannins, flavonoids, and glycosides which influence many physiological and pathological conditions¹⁴⁻¹⁶. The steroidal saponins may justify its use as general tonic and against hypoglycemia, hypolipidemia and several other ailments¹⁷⁻¹⁹. It has also been reported to protect against oxidative stress, heavy metal toxicity, cytotoxicity, fungal and helminthic infections^{13, 20}. Beneficial effects of this plant though have been investigated on many systems, but no reference is available with respect to gut ailments.

In view of this, the fruit extract of Tt was evaluated *in vitro* for antioxidant (DPPH), anti-inflammatory (anti-denaturation of protein), antitumor (MTT assay) in HCT (human colorectal tumor) cells. Activity of Tt was also investigated with respect to antimicrobial action (Zone of inhibition, ZI) and growth profile of *Lactobacillus rhamnosus* (*L.rh*).

Materials and Methods

Reagents

Analytical grade chemicals like 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and acetone were procured from M/s Qualigens (India). Dulbecco's modified Eagle medium (DMEM), MRS (DeMan Rogosa and Sharpe), EMB (eosin methylene blue), Nutrient Broth, Fetal bovine serum (FBS), Trypsin 0.25%, antibiotic solution and trypan blue were procured from M/s Hi-media (India). Methanol and ethanol were procured from Merck (Mumbai); Indomethacin from MP Biomedicals (Strasbourg, France) and Quercetin from Sisco Research Laboratories (Mumbai).

Microbial culture

Inoculum of culture of *Lactobacillus rhamnosus* was procured from Microbial Type Culture Collection and Gene bank (MTCC), Chandigarh and *Escherichia coli* from the Department of Biosciences, Jamia Millia

Islamia, New Delhi and *L. rhamnosus* was grown on MRS and *E. coli* on EMB (selective growth media) at 37°C.

Mammalian cell culture

Human colorectal carcinoma cell line HCT-15 was procured from the National Centre for Cell Sciences (NCCS), Pune and grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 µg/mL) and maintained at 5% CO₂ in incubator at 37°C.

Preparation of *T. terrestris* fruit extract

The dried fruits of *T. terrestris* collected from 'local dealer for medicinal herbs' at Khari Baoli, Old Delhi, were powdered using a mixer grinder. About 10 g of this powder was mixed with 100 mL of a mixture of absolute ethyl alcohol and distilled water (50:50 v/v) and kept for 24 h on magnetic stirrer for continuous stirring. After 24 h, the homogenate was filtered through a muslin cloth and thereafter through 0.22 µ membrane filter assembly. The filtrate was concentrated using rota-vapour and was finally stored at 4°C.

Assessment of antioxidant activity (DPPH assay)

Antioxidant potential of Tt fruit extract was estimated by radical scavenging activity against stable DPPH free radicals following the method described by Shalaby and Shanab²¹. Definite volume (25-800 µL) of the extract was taken in the test tubes and double distilled water was added to make it to 1.0 mL. The 2.0 mL of freshly prepared DPPH solution (0.1 mM in methanol) was added to each test tube, mixed properly and incubated in a dark room at ambient temperature for 30 min. The absorbance of the mixture was measured at 517 nm using double beam spectrophotometer (Systronics). The experiments were carried out in triplicate and per cent inhibition of DPPH radical was calculated as per the following formula.

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100,$$

where A₀ referred to the absorbance of the control and A₁ to the test sample.

Evaluation of anti-inflammatory activity

Anti-Inflammatory activity of Tt fruit extract was estimated by inhibition of protein denaturation activity as described by Sen *et al.*²². The reaction mixture (5 mL) consisted of 0.2 mL of fresh egg albumin (hen's egg); 2.8 mL of phosphate buffered

saline (PBS, pH 6.4) and 2 mL of varying concentrations of the Tt extract, making final solution with concentrations ranging from 10 to 320 µg/mL. Similar volume of double-distilled water served as control. The mixtures were incubated at 37°C for 15 min and heated at 70°C for 5 min and were allowed to cool at ambient temperature. Thereafter, the absorbance was measured at 660 nm. Indomethacin at the corresponding concentration was used as a reference drug. The percentage of inhibition of protein denaturation by the extract was calculated using the following formula:

$$\% \text{ inhibition} = \{(V_t / V_c) - 1\} \times 100;$$

where, V_t = absorbance of test sample, and V_c = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined from the dose response curve by plotting percentage inhibition against treatment concentration.

Cytotoxic effects (MTT assay)

Antiproliferative and cytotoxic effects of Tt fruit extract on carcinoma cell line (HCT-15) were studied using MTT assay as described by Mathew and Subramanian²³. Cells were plated in triplicate at a density of 5×10^3 cells/well each having 200 µL culture medium in a 96-well plate. Cells were treated with 100 µL of increasing concentrations of Tt fruit extract (10-70 µg/mL) and cultured for additional 24 h. After this, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and the cells were incubated at 37°C for 4 h to allow MTT to be metabolized. Media was then discarded and the plate was allowed to dry. The resulting formazan crystals in each well were solubilized by the addition of 200 µL DMSO. The absorbance was measured at 570 nm.

Anti-microbial potential of Tt against *E. coli*

Antimicrobial potential of Tt was assessed by well diffusion assay by measuring area of Zone of Inhibition (ZI) as described by Holder and Boyce²⁴. Soft nutrient agar plates were prepared using petri dishes of 90 mm diameter and *E. coli* was seeded as test organism. Wells of equal size (10 mm in diameter) were bored at the center of each agar plate. Varying concentration of Tt fruit extract (100 to 1000 µg/mL) in 1 mL final volume was poured in each well. Plates were incubated at 37°C for 24 h. Thereafter, zone of inhibition was measured.

Effect of Tt on growth of *Lactobacillus rhamnosus*

The effect of fruit extract of *Tribulus terrestris* (Tt) was investigated on growth-dynamics of probiotic *L. rhamnosus* (*L.rh*) by measuring optical density (OD) at 600 nm as described by Miao et al.²⁵. The *L.rh* were multiplied in MRS broth for 24 h, and thereafter used for inoculating various test flasks containing fresh media. Culture inoculum of *L.rh* (1×10^8 bacterial cells in 500 µL growth media) was added to each flask containing 25 mL of growth media with or without Tt extract (different concentrations ranging 50 to 250 µg/mL). All the flasks were incubated at 37°C. Density of *L.rh* in each flask was estimated at regular time intervals by measuring absorbance at 600 nm.

Statistical analysis

The data of at least three experiments, each conducted separately was pooled together and was subjected to statistical analysis. The results have been presented in the form of Mean plus Standard Error (MSE). Student t-test was applied to compare the effect of control and treated groups. $P < 0.05$ was considered as statistically significant.

Results and Discussion

The fruit extract of *Tribulus terrestris* (Tt) was extracted using aqua-alcoholic solvent (50:50, v/v). Aqueous or methanolic extracts of *T. terrestris* fruits exhibited comparable antibacterial activity²⁶. Hence, the solvent used for extraction of Tt is really not very vital though some solvents may be more effective for some solutes than others.

Antioxidant effects of Tt extract (% Inhibition of DPPH radicals)

Increase in Tt concentration from 25 to 800 µg/mL displayed a rising trend of inhibition of DPPH radicals from 29.5 to 84 % (Table 1). Similarly, increasing concentration of quercetin from 20 to 120 µg/mL enhanced inhibition of DPPH radicals from 41.8 to 99.1%, respectively (Table 1). IC_{50} values of Tt and quercetin with respect to DPPH radical scavenging were calculated as 98.83 and 24.77 µg/mL, respectively.

The ROS induced oxidative damage to the cells and tissues is known to lead to the genesis of a large number of diseases. Tt induced inhibition remained concentration dependent as is known for most of the biomolecules. Quercetin, a well known antioxidant biomolecule was taken as a reference compound to assess comparative efficacy of Tt extract²⁷. Several flavonoids like quercetin 3-O-glycoside, quercetin 3-O-

rutinoside and kaempferol 3-O-glycoside have been reported in Tt fruit extract^{28,29}. The present study however didn't investigate the effectiveness of various quercetins present in the Tt fruit extract, but these could be studied in the future. Isolated quercetin obtained from industry, was several fold more effective than Tt extract as is evident from their IC50 values. This could be attributed to the presence of smaller number of effective DPPH radical scavenging molecules per unit volume in the whole extract of Tt. Tt extract administration before irradiation has also been reported to provide protection by inhibiting radiation induced glutathione depletion and decreasing lipid-peroxidation in the liver of mice³⁰. The spectrum of antioxidant activities displayed by the Tt fruit extract generates a strong basis for its utility against oxidative species in the gut where metabolic activities of a large number of microbes and their interaction with gut tissues and diet generate enormous oxidative activities. It is interesting to note that most of the antioxidants like ascorbate lose their antioxidant potential on heating but Tt retains its antioxidant activity even during cooking³¹. This property of thermos-stability of Tt may prove useful for preparing many ayurvedic medicines which undergo extensive heating during manufacturing processes.

Anti-inflammatory effects of Tt (% Inhibition of protein denaturation)

The increasing concentration of indomethacin and Tt fruit extract rendered inhibitory effect on

denaturation of protein in a concentration dependent manner (Table 2). However, the effect of Tt fruit extract was more pronounced than indomethacin at each corresponding concentration. This effect of Tt was several folds greater at higher concentrations. IC₅₀ for indomethacin and Tt extract were calculated as 12.9 and 10.8 µg/mL, respectively.

Inflammation is an important parameter that precedes development of many diseases. In the present study, the protein denaturation bioassay³² was used for *in vitro* assessment of anti-inflammatory activity of Tt fruit extract³³. During the present study, the Tt fruit extract rendered inhibitory effect on denaturation of protein in a concentration dependent manner (Table 2). Our results corroborated the findings of Oh *et al.*³⁴, who reported inhibition of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in lipopolysaccharide induced inflammaed cells (RAW264.7) by ethanolic extract of Tt. Similarly, the methanolic extract of Tt was demonstrated to render dose-dependent inhibition of rat paw volume in carrageenan induced inflammation³⁵. Therefore, the extraction medium whether ethanolic or methanolic, didn't reveal any appreciable difference on the anti-inflammatory potential of Tt. Indomethacin (NSAID), taken as a reference for comparison, also displayed concentration based denaturation of proteins. However, the effects of Tt extract were more pronounced than indomethacin as reflected by the

Table 1 — Anti-oxidant effect (% Inhibition of DPPH radicals) of *Tribulus terrestris* fruit extract at different concentrations was compared with quercetin which was taken as a reference. Tt or Quercetin solutions indicated their amount in 1 mL of test solution added to 2 mL of DPPH solution. Per cent inhibition of DPPH radicals was computed using the formula $(A_0 - A_1 / A_0) \times 100$, where, A₀ = absorbance of the control and A₁ = absorbance of the test sample.

<i>Tribulus terrestris</i> (µg)	(% Inhibition of DPPH radicals)	Quercetin (µg)	(% Inhibition of DPPH radicals)
Control (untreated)	-	Control (untreated)	-
25	29.54 ^a ± 1.076	20	41.76 ^a ± 0.248
50	36.50 ^b ± 0.970	40	75.96 ^b ± 0.232
100	44.44 ^c ± 0.615	60	90.22 ^c ± 0.414
200	65.00 ^d ± 0.409	80	94.25 ^d ± 0.370
400	79.22 ^e ± 0.729	100	97.7 ^e ± 0.334
800	84.11 ^f ± 0.700	120	99.08 ^e ± 0.185

[Each value represented a mean of three experiments and was expressed as Mean ± SE. Each concentration was ascribed with a superscript (a-f) indicating statistical significance (P <0.05)]

Table 2 — Anti-inflammatory effect (% inhibition of protein denaturation) of *Tribulus terrestris* fruit extract at different concentrations was compared with indomethacin which was taken as a reference. Per cent inhibition of protein denaturation was calculated as $\{(V_t / V_c) - 1\} \times 100$; where, V_t and V_c represented the absorbance of the test and control respectively.

Concentration (µg/mL)	% Inhibition of Protein denaturation	
	Indomethacin (IM)	<i>Tribulus terrestris</i> (Tt)
Control	-	-
10	11.28 ± 1.36	27.18* ± 3.12
20	141.03 ± 4.01	270.77** ± 4.94
40	288.72 ± 5.05	890.77*** ± 4.07
80	455.38 ± 4.94	1199.49*** ± 5.43
160	1047.18 ± 6.89	2421.02*** ± 6.24
320	1577.44 ± 4.47	3257.44*** ± 3.12

[Each value represented a mean of three experiments and was expressed as Mean ± SE. Statistical significance was determined using t-test and alphabets shown on each value as a superscript indicated the level of significance (* P <0.05, ** P <0.01 and *** P <0.001)]

IC₅₀ values. The higher anti-inflammatory effect of Tt fruit extract with respect to indomethacin can be attributed to the synergistic interaction of the presence of large number of biomolecules like alkaloids³⁶ and flavonoids.

Antitumor effects of Tt extract on HCT-15 cells

The results of MTT assay conducted on HCT-15 cells have been displayed in Fig. 1. The concentration of 10 and 20 µg/mL of Tt fruit extract rendered a slight increase in cell viability in comparison to the control, however this change was statistically insignificant. Higher concentration like 30 µg/mL of Tt extract decreased the cell viability significantly (~78%). Further increase in concentration of Tt fruit extract at 40 µg/mL and beyond decreased the cell viability to about 29 to 22 % (a plateau like response at 50-70 µg/mL).

Our results corroborated with the findings of Bedir *et al.*³⁷ about the cytotoxicity of Tt. There are a number of saponins which may account for the antitumor effect in Tt fruit extract. Kim *et al.*³⁸ demonstrated enhanced cell growth arrest and apoptosis in HepG2 cells by downregulating NFκB signaling. However, there is no report available on cytotoxic effects of Tt on intestinal epithelial cells. Under normal condition, the intestinal epithelium is protected by a covering of mucus layer. However, the cancerous portion of epithelium is deficient of protective mucus layer and is therefore more prone to the action of Tt. The pathogenic bacteria are already under stress because of the bacteriocin secreted by the probiotic bacteria which largely outnumber the pathogenic forms. The presence of active antimicrobial molecule of Tt may synergize the action of bacteriocin against pathogenic microbes. However, this difference could be further investigated. Our study reports possibly the first time the cytotoxic effect of Tt on HCT-15 cells, a carcinoma cell line of the intestine. This could be a basis for its application in the development of a therapeutic agent against diseases like polyps, intestinal tumors, etc.

Antimicrobial potential of Tt extract against *E. coli*

The results of well diffusion assay revealing the zone of inhibition on the growth of *E. coli* have been displayed in Fig. 2. Concentration of Tt up to 200 µg/mL did not significantly change ZI, but higher doses ranging from 400 to 1000 µg/mL increased ZI from 3.37±0.293 cm² to 9.82±0.036 cm².

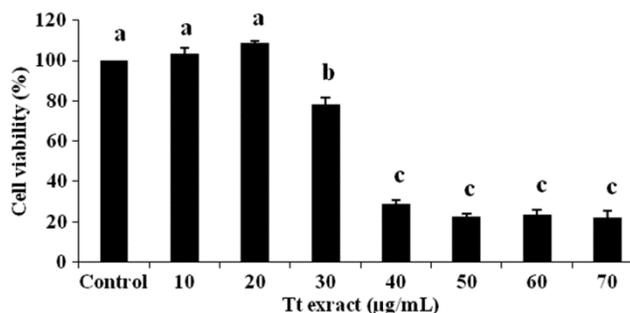


Fig. 1 — Cytotoxic effect of different concentrations of *Tribulus terrestris* on HCT-15 (colorectal carcinoma) cells was evaluated by per cent change in cell viability considering viability of control as 100 %. [Each bar represented the Mean ± SE of three experiments. Different alphabets (a to c) above each bar indicates significant differences among mean values at $P < 0.05$ and similar letters indicated non-significant differences among mean values]

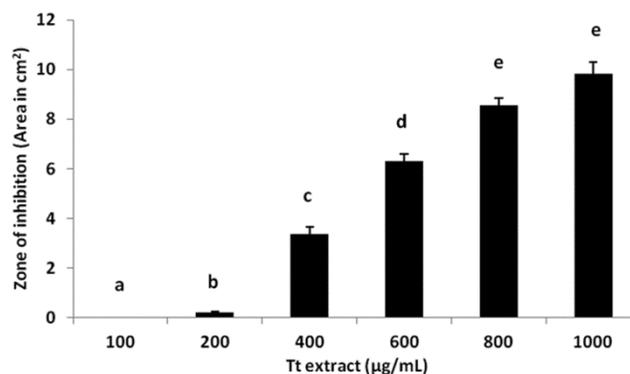


Fig. 2 — Anti-microbial potential of *Tribulus terrestris* at different concentrations against *E. coli*. [Zone of Inhibition (ZI) indicates the clear zone showing no bacterial (*E. coli*) growth. Each bar represented the Mean ± SE of three experiments. Different alphabets (a to e) above each bar indicated significant differences among mean values at $P < 0.05$ and similar letters indicated non-significant differences among mean values]

Tt has been reported by Bedir *et al.*³⁷ and Zhang *et al.*³⁹ to render cytotoxic activity against *Candida albicans* and *Cryptococcus neoformans*, respectively. Gomathi *et al.*²⁹ had reported the antimicrobial activity of Tt against both gram positive and Gram negative bacteria. These studies indicated the presence of broad-spectrum antimicrobial, antifungal and antitumor molecules in Tt fruit extract.

Effect of Tt fruit extract on growth of *L.rh*

The growth of *L.rh* (control) without any administration of Tt fruit extract remained in log phase from 4 to 16 h of incubation and followed a stationary phase thereafter up to 24 h (Fig. 3). The *L.rh* cultures treated with various concentrations of Tt for 4 h did not display any growth over the control. However, at 8 h, an increase in growth was observed especially with the treatment of 150 and

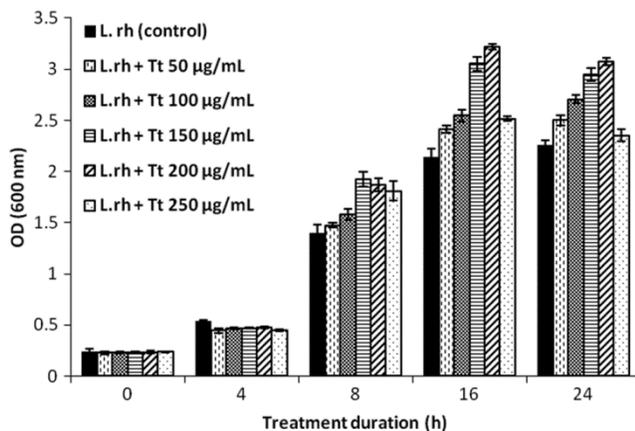


Fig. 3 — Effect of different concentrations of *Tribulus terrestris* (Tt) on growth dynamics of *Lactobacillus rhamnosus* (L.rh) evaluated by measuring OD at 600 nm at different time intervals. [Each bar represented the Mean \pm SE of three experiments. The 150 and 200 $\mu\text{g/mL}$ treated groups were significantly different ($P < 0.05$ and $P < 0.01$) from untreated (control) groups at 8 and 16 h, respectively. At 24 h, 100, 150 and 200 $\mu\text{g/mL}$ treated groups were significantly different ($P < 0.05$) from untreated (control) groups]

200 $\mu\text{g/mL}$ Tt fruit extract (OD-1.93 and 1.87, respectively) over the control (OD-1.40), and at 16 h the growth of *L.rh* was significantly enhanced (OD-3.05 and 3.22, respectively) over the control (OD-2.14). At 24 h the growth of *L.rh* did not show a significant enhancement over the 16 h treatment group. The highest concentration of Tt used here (250 $\mu\text{g/mL}$) did not show a significant enhancement in the growth of *L.rh* over the control at any treatment duration. Our results corroborated the findings of Chen *et al.*⁴⁰, who reported similar effects of Tt on the growth of *Lactobacillus acidophilus*.

Conclusion

Antioxidant and anti-inflammatory properties of *Tribulus terrestris* fruit extract (Tt) and its cytotoxic action against human colorectal carcinoma, HCT-15 cell line can be exploited against several pathological conditions in the gut. The selective anti-microbial effects of Tt against pathogenic *Escherichia coli* and growth enhancement of *Lactobacillus rhamnosus* further strengthens its therapeutic potential against gut ailments which are generated due to imbalances of gut microbial populations.

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Conflict of interest:

There is no conflict of interest among the authors.

References

- 1 Hoivik ML, Moum B, Solberg IC, Henriksen M, Cvancarova M & Bernklev T, Work disability in inflammatory bowel disease patients 10 years after disease onset: results from the IBSEN Study. *Gut*, 62 (2013) 368.
- 2 Hulisz D, The burden of illness of irritable bowel syndrome: current challenges and hope for the future. *J Manag Care Pharm*, 10 (2004) 299.
- 3 Geneau R, Stuckler D, Stachenko S, Mckee M, Ebrahim S, Basu S, Chockalingham A, Mwatsama M, Jamal R, Alwan A & Beaglehole R, Raising the priority of preventing chronic diseases: a political process. *Lancet*, 376 (2010) 1689.
- 4 Sun J & Chang EB, Exploring gut microbes in human health and disease: pushing the envelope. *Genes Dis*, 1 (2014) 132.
- 5 Conlon MA & Bird AR, The Impact of diet and lifestyle on gut microbiota and human health. *Nutrients*, 7 (2015) 17.
- 6 Alzogaibi M, Concepts of oxidative stress and anti-oxidant defense in Crohn's disease. *World J Gastroenterol*, 19 (2013) 6540.
- 7 Tatay IM, Iborra M, Cerrillo E, Tortosa L, Nos P & Beltran B, Possible biomarkers in blood for crohn's disease: oxidative stress and microRNAs—current evidences and further aspects to unravel. *Oxid Med Cell, Longev*, 28 (2016) 1.
- 8 Rana V, Sharma S, Prasad KK, Sinha SK & Singh K, Role of oxidative stress & anti-oxidant defence in ulcerative colitis patients from north India. *Indian J Med Res*, 139 (2014) 568.
- 9 Li H, Wu Q, Xu L, Li X, Duan J, Zhan J, Feng J, Sun X & Chen H, Increased oxidative stress and disrupted small intestinal tight junctions in cigarette smoke-exposed rats. *Mol Med Rep*, 11 (2015) 4639.
- 10 Reuter S, Gupta SC, Chaturvedi MM & Aggarwal BB, "Oxidative stress, inflammation, and cancer: how are they linked?" *Free Radic Biol Med*, 49 (2010) 1603.
- 11 Schlansky B & Hwang JH, Prevention of nonsteroidal anti-inflammatory drug-induced gastropathy. *J Gastroenterol*, 19 (2009) 44.
- 12 Rizwan M & Khan AA, Assessment of efficacy of Sankhahuli (*Convolvulus pluricaulis* C) and gokhru (*Tribulus terrestris* L.) in the management of hypertension. *Indian J Tradit Knowle*, 13 (2014) 313.
- 13 Chhatre S, Nesari T, Somani G, Kanchan D & Sathaye S, Phytopharmacological overview of *Tribulus terrestris*. *Pharmacogn Rev*, 8 (2014) 45.
- 14 Kostova I & Dinchev D, Saponins in *Tribulus terrestris*—chemistry and bioactivity. *Phytochem Rev*, 4 (2005) 111.
- 15 Wu TS, Shi LS & Kuo SC, Alkaloids and other constituents from *Tribulus terrestris*. *Phytochemistry*, 50 (1999) 1411.
- 16 Mitra N, Mohammad MD & Mohammad RZ, *Tribulus terrestris* L. (Zygophyllaceae) Flavonoid Compounds. *Int J Mod Bot*, 2 (2012) 35.
- 17 Khan S, Kabir H, Jalees F, Asif M & Naquvi KJ, Antihyperlipidemic potential of fruits of *Tribulus terrestris* Linn. *Int J Biomed Res*, 2 (2011) 98.
- 18 Samani NB, Jokar A, Soveid M, Heydari M & Mosavat SH, Efficacy of the hydroalcoholic extract of *Tribulus terrestris*

- on the serum glucose and lipid profile of women with diabetes mellitus: A double-blind randomized placebo-controlled clinical trial. *J Evid Based Complementary Altern Med*, 21 (2016) 91.
- 19 El-Tantawy WH & Hassanin LA, Hypoglycemic and hypolipidemic effects of alcoholic extract of *Tribulus alatus* in streptozotocin- induced diabetic rats: a comparative study with *T. terrestris* (Caltrop). *Indian J Exp Biol*, 45 (2007) 785.
 - 20 Lakshmi GD, Kumar PR, Bharavi K, Annapurna P, Rajendar B, Patel PT, Satish CSV & Rao GS, Protective effect of *Tribulus terrestris* L. on liver and kidney in cadmium intoxicated rats. *Indian J Exp Biol*, 50 (2012) 141.
 - 21 Shalaby EA & Shanab SM, Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extract of *Spirulina platensis*. *Indian J Geo Mar Sci*, 42 (2013) 556.
 - 22 Sen S, Chakraborty R, Maramsa N, Basak M, Deka S & Dey BK, *In vitro* anti-inflammatory activity of *Amaranthus caudatus* L leaves. *Indian J Nat Prod Resour*, 6 (2015) 326.
 - 23 Mathew M & Subramanian S, *In vitro* evaluation of anti-alzheimer effects of dry ginger (*Zingiber officinale* Roscoe) extract. *Indian J Exp Biol*, 52 (2014) 606.
 - 24 Holder IA & Boyce ST, Agar well diffusion assay testing of bacterial susceptibility to various anti-microbials in concentrations non-toxic for human cells in culture. *Burns*, 20 (1994) 426.
 - 25 Miao Z, Zhang Y, Wang D & Li Z, Effect of polysaccharides and water extract from *Fructus tribuli* on growth of *Lactobacillus brevis*. *Adv J Food Sci Technol*, 6 (2014) 72.
 - 26 Sharma M, Kumar A, Sharma B, & Dwivedi N, Evaluation of phytochemical compounds and anti-microbial activity of leaves and fruits *Tribulus terrestris*. *Europ J Exp Biol*, 3 (2013) 432.
 - 27 Majewska M, Skrzycki M, Podsiad M & Czczot H, Evaluation of antioxidant potential of flavonoids: an *in vitro* study. *Acta Pol Pharm*, 68 (2011) 611.
 - 28 Yekta MM, Alavi SHR, Hadjiaghaee R, & Ajani Y, Flavonoid Glycosides from *Tribulus terrestris* L. orientalis. *Iranian J Pharmaceut Sci*, 4 (2008) 231.
 - 29 Gomathi S, Shanmugapriya A, Bharathi V, Gayathri G & Karpagam T, Anti-microbial Activity and Phytochemical Studies of Aqueous and Ethanolic Fruit Extracts of *Tribulus terrestris*. *IJPI'S J Pharm Herb Formul*, 2 (2012) 47.
 - 30 Kumar M, Panwar M, Samarth R & Kumar A, Evaluation of radiomodulatory influence of *Tribulus terrestris* root extract against gamma radiation: hematological, biochemical and cytogenetic alterations in swiss albino mice. *Pharmacologyonline*, 1 (2009) 1214.
 - 31 Sasikala S, Kannan E & Brindha D, Qualitative characterization of solvent and cooked extracts of *Tribulus terrestris* L. Fruit. *Europ J Med Plants*, 4 (2014) 907.
 - 32 Williams LAD, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, Conrad J, Vogler B, Rosner H & Kraus W, The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J*, 57 (2008) 327.
 - 33 Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN & Iputp JE, An experimental evaluation of *Albucca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *J Med Plants Res*, 4 (2010) 789.
 - 34 Oh JS, Baik SH, Ahn EK, Jeong W & Hong SS, Anti-inflammatory activity of *Tribulus terrestris* in RAW264.7 Cells. *J Immunol*, 188 (2012) 54.
 - 35 Baburao B, Rajyalakshmi G, Venkatesham A, Kiran G, Sunder A & Rao BG, Anti-inflammatory and anti-microbial Activities of methanolic extract of *Tribulus terrestris* L. plant. *Int J Chem Sci*, 7 (2009) 1867.
 - 36 Souto AL, Tavares JF & da Silva MS, Anti-Inflammatory Activity of Alkaloids: An Update from 2000 to 2010. *Molecules*, 16 (2011) 8515.
 - 37 Bedir E, Khan IA, & Walker LA, Biologically active steroidal glycosides from *Tribulus terrestris*. *Pharmazie*, 57 (2002) 491.
 - 38 Kim HJ, Kim JC, Min JS, Kim MJ, Kim JA, Kor MH, Yoo HS & Ahn JK, Aqueous extract of *Tribulus terrestris* L induces cell growth arrest and apoptosis by down-regulating NF- κ B signaling in liver cancer cells. *J Ethnopharmacol*, 136 (2011) 197.
 - 39 Zhang JD, Xu Z, Cao YB, Chen HS, Yan L, An MM, Gao PH, Wang Y, Jia XM & Jian YY, Antifungal activities and action mechanisms of compounds from *Tribulus terrestris* L. *J Ethnopharmacol*, 103 (2006) 76.
 - 40 Chen H, Qin T, Ma Q, Ji Z & Shu G, Effect of the steroidal saponins of *Fructus tribuli* on growth of three *Lactobacillus acidophilus*. *Adv J Food Sci Technol*, 6 (2014) 135.