Publications

Publications in International and National Journal:

From the Thesis:

1. **Hussain A** and Ramteke A **(2012)** Flower Extract *of Nyctanthes arbortristis* Modulates the Glutathione level in Hydrogen Peroxide treated lymphocytes**.** ***Pharmacognosy Research;*** **4**(4): 230-233. DOI: 10.4103/0974-8490.102272 [ISSN No: 0976-4836 (Print), 0974-8490 (Online)]

2. **Hussain A**, Saikia V and Ramteke A **(2012)** Nitric Oxide and Modulatory Effects of the Root extract of *Phlogacanthus tubiflorus* against Oxidative Stress induced by Hydrogen Peroxide. ***Free Radical and Antioxidant;*** **2**(1): 9-12. DOI: 10.5530/ax.2012.2.4 [ISSN No: 2231-2536]

3. Ramteke A, **Hussain A**, Kaundal S and Kumar G **(2012)** Oxidative Stress and Modulatory effects of the root extract of *Phlogacanthus tubiflorus* on the activity of Glutathione-*S*-Transferase in Hydrogen Peroxide treated Lymphocyte. ***Journal of Stress Physiology & Biochemistry;*** **8**(1): 5-15. [ISSN No: 1997-0838]

Others :

4. Deep G, Kumar R, Jain A K, Dhar D, Panigrahi G K, **Hussain A**, Agarwal C, El-Elimat T, Sica V P, Oberlies N H and Agarwal R (2016) Graviola inhibits hypoxia-induced NADPH oxidase activity in prostate cancer cells reducing their proliferation and clonogenicity. ***Scientific Reports***; 6: 23135. DOI: 10.1038/srep23135 [ISSN No: 2045-2322]

5. Banik N, **Hussain A**, Ramteke A and Maji T K **(2015)** Synthesis and Evaluation of the Properties of Chitosan-Cellulose Whisker Microparticles for Controlled Release of Isoniazid. ***American Journal of Polymer Science & Engineering;*** **3**(1): 100-118.

6. Saikia C, **Hussain A**, Ramteke A, Sharma H K, Deb P and Maji T K **(2015)** Carboxymethyl starch-coated iron oxide magnetic nanoparticles: a potential drug delivery system for isoniazid. ***Iranian Polymer Journal;*** **24**(10): 815-828. DOI: 10.1007/s13726-015-0370-z [ISSN No: 1026-1265 (Print), 1735-5265 (Online)]

7. Sarmah M, Banik N, **Hussain A**, Ramteke A, Sharma H K and Maji T K **(2015)** Study on crosslinked gelatin-montmorillonite nanoparticles for controlled drug delivery applications. ***Journal of Materials Science;*** **50**(22): 7303-7313. DOI: 10.1007/s10853-015-9287-3 [ISSN No: 0022-2461 (Print), 1573-4803 (Online)]

8. Saikia C, **Hussain A**, Ramteke A, Sharma H K and Maji T K **(2014)** Carboxymethyl starch-Chitosan coated iron oxide magnetic nanoparticles for controlled delivery of isoniazid. ***Journal of Microencapsulation;*** **32**(1): 29-39. DOI: 10.3109/02652048.2014.940015 [ISSN No: 0265-2024 (Print), 1464-5246 (Online)]

9. Saikia C, **Hussain A**, Ramteke A, Sharma H K and Maji T K **(2014)** Crosslinked Thiolated Starch (TS) coated Fe_3O_4 magnetic nanoparticles: Effect of MMT and crosslinking density on controlled drug delivery properties. ***Starch;*** **66**(7-8): 760-771. DOI: 10.1002/star.201300277 [ISSN No: 0038-9056 (Print), 1521-379X (Online)]
10. Sarma R, Das Q, **Hussain A**, Ramtake A, Mohanta D and Choudhury A J **(2014)** Physical and biophysical assessment of highly fluorescent, magnetic quantum dots of wurtzite-phase manganese selenide system. ***Nanotechnology;*** **25**(27): 275101. DOI: 10.1088/0957-4484/25/27/275101 [ISSN No: 0957-4484 (Print), 1361-6528 (Online)]
11. Ramteke A, Ting H, Agarwal C, Mateen S, Somasagaral R, **Hussain A**, Graner M, Frederick B, Agarwal R and Deep G **(2013)** Exosomes Secreted under Hypoxia Enhance Invasiveness and Stemness of Prostate Cancer Cells by Targeting Adherens Junction Molecules. ***Molecular Carcinogenesis;*** **54**(7): 554-565. DOI: 10.1002/mc.22124 [ISSN No: 0899-1987 (Print), 1098-2744 (Online)]
12. Banik N, Iman M, **Hussain A**, Ramteke A, Boruah R and Maji T K **(2013)** Soy flour Nanoparticles for Controlled Drug Delivery: Effect of Crosslinker and Montmorillonite (MMT). ***New Journal of Chemistry;*** **37**(12): 3981-3990. DOI: 10.1039/C3NJ00480E [ISSN No: 1144-0546 (Print), 1369-9261 (Online)]
13. **Hussain A**, Zaman M K and Ramteke A **(2013**) Preliminary Phytochemical Screening and Proximate Analysis of the trunk bark of *Alstonia scholaris* (L.) R.Br. ***Journal of Pharmacognosy and Phytochemistry;*** **1(**5): 13-17. [ISSN No: 2349-8234 (Print), 2278-4136 (Online)]
14. Banik N, **Hussain A**, Ramteke A, Sharma H and Maji T K **(2012)** Preparation and evaluation of the effect of particle size on the properties of Chitosan-Montmorillonite nanoparticle loaded with Isoniazid. ***RSC Advances;*** **2**: 10519-10528. DOI: 10.1039/C2RA20702H [ISSN No: 2046-2069]

Book chapter:

15. Saikia V, **Hussain A** and Ramteke A **(2015)** Screening, biochemical characterization and identification of metal resistant bacterial strains isolated from adjoining areas of mining sites, In: Konwar D and Kalita P (Eds), ***Molecular Biology and Biotechnology Research in NE India***, Pub Kamrup College, Baihata Chariali, Kamrup, Assam, India, 43-54 [ISBN No: 978-81-931525-0-8]

Paper presented in International and National Conferences/seminar/Symposia:

1. Gumpricht E, Kumar R, **Hussain A**, Sabarwal A, Ramteke A, Cho S and Deep G **(2015)** Natural Herbal Beverage Exhibits Significant Cytoprotection and Promotes Nrf-2 Activation in Cells, ***Experimental Biology-2015***, held at Boston Convention and Exhibition Centre, Boston (USA) on 28th March-1st April, 2015. **[Abstract published in *The FASEB Journal*, 2015; 29: 1 Supplement 607.1]** [ISSN No: 0892-6638 (Print), 1530-6860 (Online)]

2. **Hussain A**, Das M K, Baishya P and Ramteke A **(2015)** Leaf extract of *Tita bahok* exhibits significant antioxidant and promotes protective function against H_2O_2 induced oxidative stress, ***International Symposium on "Current Advances in Radiobiology, Stem cells and Cancer Research***, held at Jawaharlal Nehru University, New Delhi, (India) on 19-21st February, 2015.

3. **Hussain A**, Tiku A B and **Ramteke A (2013)** *Nyctanthes arbortristis* modulates ROS, Xenobiotic metabolising enzymes and antioxidants to inhibit murine skin tumorigenesis, ***5th HOPE Meeting with Noble Laureates*** organized by Japan Society for the Promotion of Science (JSPS), Tokyo, Japan on 26th February to 2nd March, 2013.

4. **Hussain A**, Saikia V, Gogoi B and Ramteke A (2012), Bark Extract of *Alstonia scholaris* modulates Nitric Oxide (NO) levels in Hydrogen Peroxide treated Lymphocytes, ***International Symposium on Recent Advances in Cancer Research: Therapeutics to Chemoprevention*** held at Central University of Gujarat, Gujarat (India) on 8-9th February, 2012.

5. **Hussain A**, Tiku A B and Ramteke A **(2012)** Nitric Oxide modulatory activity of *Nyctanthes arbortristis* flower extract: *in vitro* and *in vivo* study, ***International Conference on Emerging Frontiers & Challenges in Radiation Biology***, held at Government Dungar College, Bikaner, Rajasthan (India) on 24-25th January, 2012

6. **Hussain A**, Tiku A B and Ramteke A **(2012)** Antioxidative effects of *Nyctanthes arbortristis* flower extract: *in vitro* and *in vivo* study, ***International Conference on Cancer Prevention, Diagnosis & Treatment*** held at University of Rajasthan, Jaipur, Rajasthan (India) on 21-22nd January, 2012.

7. **Hussain A**, Gogoi B and Ramteke A **(2011),** Oxidative stress and modulatory effects of the flower extracts of *Nyctanthes arbortristis* on the activity of Glutathione-S-transferase , ***International Cancer Conference on Recent Advances in Cancer Research: Bench-to-Bedside*** held at Central University of Gujarat, Gujarat (India) on 19-20th February 2011.

8. **Hussain A**, Kaundal S, Devi Y P and Ramteke A **(2011)** Oxidative stress and modulatory effects of the root extracts of *Phlogacanthus tubiflorus* on the activity of Glutathione-S-transferase , ***30th Annual Convention of Indian Association for cancer research & International Symposium on "Signaling Network and Cancer"*** held at IICB, Kolkata (India) on 6 -9 February 2011.

9. **Hussain A**, Kumar G, Gogoi B and Ramteke A **(2011)** Nitric Oxide and Modulatory Effects of the Root extracts of *Phlogacanthus tubiflorus* against Oxidative Stress induced by Hydrogen Peroxide, ***30th Annual Convention of Indian Association for cancer research & International Symposium on "Signaling Network and Cancer"*** held at IICB, Kolkata (India) on 6 -9 February 2011.

10. Pathak S S, Sahay S, **Hussain A**, Saikia V and Ramteke A **(2010)** Comparative Genomic Analysis of *Deinococcus radiodurans*: An evolutionary Prospective, ***National Conference on Biotechnology for all*** held at North Maharashtra University, Jalgaon, and Maharashtra (India) on 29-30 December, 2010.

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Reprint of Publications

Flower extract of *Nyctanthes arbor-tristis* modulates glutathione level in hydrogen peroxide treated lymphocytes

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ABSTRACT

Background: *Nyctanthes arbor-tristis* Linn (Oleaceae) is a well-known traditional medicinal plant used throughout the India as an herbal remedy for treating various infectious and non-infectious diseases. **Objective:** To evaluate the antioxidative activity of hydro-alcoholic extract of flower in the lymphocytes exposed to oxidative stress induced by H_2O_2. **Materials and Methods:** Isolated lymphocytes were treated *in vitro* with extract or extract + H_2O_2, and the level of reduced glutathione (GSH) as well as the activity of glutathione-*S*-transferase (GST) and lactate dehydrogenase (LDH) were measured. **Results:** Treatment of lymphocyte with flower extract (50, 100, and 200 µg/ml) significantly increased the level of GSH and decreased the activity of GST. The LDH activity measured in the cell-free medium decreased significantly. Pre-treatment of lymphocyte with flower extract protects the lymphocyte from the H_2O_2 induced oxidative stress by significantly increasing the levels of GSH as compared to the cells treated only with H_2O_2. Pre-treatment also reduced the activity of LDH significantly as compared to the cells treated only with H_2O_2. The LDH activity in cell-free medium is associated with membrane damage, the decreased levels of LDH activity reflects the reduced level of membrane damage due to H_2O_2. **Conclusion:** The present findings suggest the protective role of the hydro-alcoholic extracts of the flower of *Nyctanthes arbor-tristis* against membrane damage induced by H_2O_2. The results also suggest that the extract might be rich in phytochemicals with antioxidant/radical scavenging potentials, which might find application in antioxidant therapy.

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INTRODUCTION

Reactive oxygen species (ROS) are generated as a metabolic by product in biological system during normal metabolism of oxygen and plays vital role in cell signaling homeostasis for maintaining normal functioning of cells.[1] In the stress conditions, either intrinsic or extrinsic, ROS levels increase dramatically, resulting imbalance in between oxidants and antioxidants that leads to various forms of damage of micro and macromolecules and finally contributes into the manifestation of disease such as sickle cell anemia, atherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's disease, schizophrenia, cancer etc.[2-5]

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Biological systems inherently have antioxidant system to scavenge and/or neutralize ROS generated under oxidative stress. Cellular antioxidant system (AOEs) consisting of mainly superoxide dismutase (SOD), catalase, reduced glutathione (GSH), glutathione peroxidase (GPx), reduced glutathione (GSH) etc.[6] These biological AOEs function as cascade manner to neutralize or eliminate the ROS and failure of which contributes the diseases manifestation. For effective management of reactive species, antioxidants have been supplemented, and several botanicals and synthetic compounds such as BHT, BHA have been studied for potent source of antioxidants. However, in real, biological state of radical scavenging and subsequent reduction of disease manifestation is still challenging area.[7]

Nyctanthes arbor-tristis Linn (commonly known as Night-flowering Jasmine), belonging to the family Oleaceae, is known for its extensive traditional medicinal use by the rural, mainly tribal people of India along with its use in Ayurveda,

Sidha, and Unani systems of medicines.[8] Traditionally, whole plants and different parts have used as an herbal remedy for treating sciatica, arthritis, malaria, enlargement of spleen and as blood purifier. The beautiful white flowers are bitter in taste and are used as stomachic, carminative, astringent to bowel, anti-bilious, expectorant, hair tonic and in the treatment of piles and various skin diseases.[9] Recent pharmacological studies showed anti-spasmodic, antioxidant, anthelmintic, cytoprotective, anti-diabetic, anti-leishmanial, CNS depressant activity of the flower extract.[9] However, very few reports are available regarding antistress or stress scavenging activity or antioxidant activity of the flower extract of this plant. Therefore, the present study was aimed to assess the modulatory response of flower extract of *Nyctanthes arbortristis* on the cellular antioxidant status in lymphocytes exposed to oxidative stress induced by hydrogen peroxide (H_2O_2) and tried to correlate with oxidative stress induced membrane damage.

MATERIALS AND METHODS

Preparation of Modulator

The flowers of *Nyctanthes arbor-tristis* were collected from healthy plants, cleaned properly, and dried at shade at room temperature. The dried plant materials were finely powdered and macerated thrice with 80% (v/v) ethanol in shaking condition for 7 days at room temperature. The extract thus obtained were filtered and concentrated by air drying and stored at 4°C. The resulting extract was dissolved in Dimethyl sulfoxide (DMSO) with final concentration of 2.5 mg/ml.

Lymphocytes Isolation, culture, and treatment

Anti-coagulated chicken blood, collected from source, was diluted with PBS (1:1, v/v), layered 6 ml into 6 ml Histopaque (1.077 gm/ml), centrifuged at 400 g for 30 minutes, and lymphocytes were isolated from the buffy layer. Isolated lymphocytes were then washed with 2 ml PBS and 2 ml RPMI media separately through centrifugation for 10 minutes at 250 g.[10,11] Pelleted lymphocytes were then suspended in RPMI, and viability was checked by Trypan blue exclusion method using hemocytometer.[12] Lymphocytes with viability more than 90% were used for subsequent study.

Isolated lymphocytes (200 µl) were seeded in 96 well culture plate in RPMI supplemented with 10% heat inactivated fetal bovine serum (FBS). Lymphocytes were treated with extract (for 4 hr), and extract+H_2O_2 (1 hr+4 hr) as per experimental requirements and maintained at 37°C and 5% CO_2 in CO_2 incubator. After incubation, lymphocytes were centrifuged and washed with PBS and homogenized in PBS. The cell homogenates were used for assaying level of GSH, GST activity, and total protein content while cell free media were used for assaying LDH activity.

Reduced glutathione (GSH) Estimation

Level of reduced glutathione was estimated as total non-protein sulphydryl group in the cell homogenates after precipitating the proteins by 5% tricholoroacetic acid (TCA). The supernatant was mixed with 0.2 M phosphate buffer (pH 8) and 0.6 M 5, 5'-dithio-bis (2-nitrobenzoic acid) and allowed to stand for 8-10 min at room temperature. The absorbance was recorded at 412 nm using a spectrophotometer (Thermo Scientific, UV 10), and level was calculated as nMole of -SH content/mg protein from standard curve made with reduced glutathione (GSH) and finally expressed as percentage change of GSH level. [13]

Glutathione-S-transferase (GST) activity

The specific activity of cytosolic GST was determined spectrophotometricaly (Cecil Aquarius, 7000 series) by measuring the CDNB-GSH conjugates formation at 340 nm for 3 min. The reaction mixture (1 ml) was prepared by mixing 0.1 M phosphate buffer (pH 6.5), 1 mM CDNB in 95% ethanol, and 1 mM GSH followed by incubation at 37°C for 5 min prior to measuring OD. The specific activity of GST was calculated using the extinction coefficient 9.6 $mM^{-1}cm^{-1}$ at 340 nm and expressed in terms of percentage change of µ mole of CDNB-GSH conjugates formed/min/mg proteins. [14]

Lactate Dehydrogenase (LDH) activity

The specific activity of lactate dehydrogenase (LDH) released into the medium was assayed by measuring the rate of oxidation of NADH at 340 nm using a spectrophometer (Cecil Aquarius, 7000 series). Briefly, assay mixture consists of 50 mM potassium phosphate buffer (pH 7.5), 0.5 mM sodium pyruvate, 0.1 mM NADH, and enzyme sample. The enzyme activity was calculated using extinction coefficient 6.22 $mM^{-1}Cm^{-1}$ and finally expressed as percentage change of LDH activity. [15]

Protein Estimation

The amounts of protein present in the sample were estimated using bovine serum albumin (BSA) as standard by using Folin reagent. [16]

Statistical Analysis

All the results are expressed as means±SD. Results were statistically analyzed by student's *t* test for significance difference between group mean using GraphPad software.

RESULTS AND DISCUSSION

The use of natural products in the form of crude preparation and active principle as a therapeutic regime has been widely established. Several medicinal plants have been studied for their potentials to modulate cellular antioxidants

and free radicals, and a numbers of active principles have also isolated from plants with anti-oxidative efficacy.[17,18] But, there is still lack of magic principle with maximum efficacy and least toxicity. The present study explores the anti-oxidative activity of flower extract of *Nyctanthes arbor-tristis*, a traditional medicinal plant of India against H_2O_2-treated lymphocytes.

H_2O_2 is weak oxidizing agent and can easily cross cell membranes and inside the cell, H_2O_2 probably reacts with Fe^{2+} and Cu^{2+} ions to forms hydroxyl radicals.[19] Increased level of hydroxyl radicals in the cell subsequently cause damage to the cells by interacting with micro and macro molecules. It also inactivate enzymes directly, usually by oxidation of essential thiol (-SH) groups. [20] Our previous study established the detrimental effects of H_2O_2. Treatment of lymphocyte with H_2O_2 decreases the viability of cells by lowering cellular antioxidant, reduced glutathione (GSH).[21]

In the present study, the level of GSH increased significantly when lymphocytes were treated with flower extract of *Nyctanthes arbor-tristis* for 4 hours, and at 200 μg/ml of treatment, 1.22-fold increase was observed in comparison to untreated lymphocytes [Table 1]. In contrast to GSH, the specific activity of glutathione-*S*-transferase, an important constituent of phase II drug metabolizing enzymes declined; however, the decline was non-significant [Table 1]. For the similar treatment condition, the specific activity of lactate dehydrogenase (LDH), marker of membrane damage was significantly declined in comparison to untreated lymphocytes [Table 1]. This decrease in the activity of LDH suggests non-toxic effect of the extract on the cellular system; rather it might have decreased the endogenous cellular injury (as a part of normal cellular metabolism).[22,23] These data clearly showed the anti-oxidative property of the crude extract used for the current study.

Increased level of cellular antioxidants is known to provide protection against oxidative stress.[24] Here, in our study, pre-treatment of lymphocytes with the flower extract (50, 100, and 200 μg/ml) for 1 h significantly restored the depleted GSH level in the 1% H_2O_2-treated lymphocytes (4 h) [Table 2]. The restoration of GSH level at 200 μg/ml treatment condition [Table 2] was above the untreated lymphocyte ($P<0.001$). As expected, the specific activity of GST decreased significantly as compared to the cells treated with H_2O_2 [Table 2]. GSH is co-factor of GST and is responsible for the redox status of cell. The rise in the levels of GSH is due to decreased GST activity.[25,26] The significant decrease in the activity of LDH was observed in the lymphocytes pre-treated with flower extract followed by H_2O_2 treatment. The significant decline in the activity of LDH suggests protective function of the extract against membrane damage induced by the H_2O_2.[27]

In conclusion, the results of the present study clearly indicate the anti-oxidative and protective role of hydro-alcoholic extract of *Nyctanthes arbor-tristis* against the oxidative stress induced by H_2O_2. The encouraging results shown by the hydro-alcoholic extract might be due to the presence of high content of phytochemicals and merits detail pharmacological investigation in suitable model to identify and characterize the active principle responsible for the observed activity.

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Table 1: Modulatory effects of the flower extract of *Nyctanthes arbor-tristis*

Treatment condition	% Change of GSH level (nMole/mg protein)	% Change of GST activity (unit/mg protein)	% Change of LDH activity (unit/mg protein)
Control	100.00±7.50	100.00±10.80	100.00±6.18
50 μg/ml extract	115.61±8.76	90.29±6.77	49.20±2.97[a]
100 μg/ml extract	125.97±9.97[c]	88.21±7.19	62.05±9.94[b]
200 μg/ml extract	122.81±9.21[c]	85.23±3.17	54.94±1.92[a]

Values are mean±SD; $n=3$; [a]$P<0.001$ compared to untreated cells; [b]$P<0.01$ compared to untreated cells; [c]$P<0.05$ compared to untreated cells

Table 2: Protective effects of flower extract of *Nyctanthes arbor-tristis* in H_2O_2 (1%) treated lymphocytes

Treatment Condition	% Change of GSH level (nMole/mg protein)	% Change of GST activity (unit/mg protein)	% Change of LDH activity (unit/mg protein)
Control	100.00±7.50	100.00±10.80	100.00±6.18
H_2O_2 (1%)	47.50±0.23[a]	168.87±8.55[a]	243.53±8.52[a]
H_2O_2 (1%)+Extract (50 μg/ml)	66.76±5.01[be]	134.66±10.10[cf]	211.10±7.39[ae]
H_2O_2 (1%)+Extract (100 μg/ml)	74.82±5.61[be]	127.99±9.60[ce]	180.59±11.98[ae]
H_2O_2 (1%)+Extract (200 μg/ml)	112.56±8.44[d]	123.83±9.29[ce]	153.29±11.41[bd]

Values are mean±SD; n=3; [a]$P<0.001$ compared to untreated cells; [b]$P<0.01$ compared to untreated cells; [c]$P<0.05$ compared to untreated cells; [d]$P<0.001$ compared to cells treated with only H_2O_2; [e]$P<0.01$ compared to cells treated with only H_2O_2; [f]$P<0.05$ compared to cells treated with only H_2O_2

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Nitric Oxide and Modulatory Effects of the Root extracts of *Phlogacanthus tubiflorus* against Oxidative Stress induced by Hydrogen Peroxide

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ABSTRACT

Introduction: Nitric oxide (NO) mediated signaling is known to influences tumor progression and has been implicated as a novel therapeutic target cancer therapy. **Methods:** In the present study, we have reported the modulatory effects of the root extracts of *Phlogacanthus tubiflorus* on NO levels in the lymphocytes cultured *in vitro* and exposed to oxidative stress induced by H_2O_2. **Results:** Increase in the levels of NO was observed in the lymphocyte treated with increasing concentration of H_2O_2 (0.1, 0.2, 0.5 and 1.0%), but the cell viability declined significantly. Treatment of Lymphocyte with root extract resulted in the decrease of NO level and increase in the Cell Viability. We also observed declined in NO levels and increase in cell viability in the lymphocyte pre-treated with the different concentrations of (50, 100, and 200 µg/ml) of root extracts and followed by the 1% H_2O_2 treatment. **Conclusion:** The present data suggests that NO and cell viability are inter related and extract used is rich in phytochemicals with modulatory effect on both NO and cell viability and hence, might find relevance in chemoprevention of oxidative stress related diseased conditions.

Key words: Chemo-modulation, Nitric Oxide, Oxidative stress, *Phlogacanthus tubiflorus*.

INTRODUCTION

Nitric oxide (NO) is highly reactive free radical capable of multitude of the reactions. It acts as an intracellular messenger and known to influence signaling pathways related to regulation of cell growth, differentiation and apoptosis and many physiological action including modulation of blood pressure and synaptic plasticity.[1] Nitric oxide also regulate hepatic metabolism and plays role in cardioprotection including regulation of blood pressure and vascular tone, inhibition of platelet aggregation and leukocyte adhesion and prevention of smooth muscle cell proliferation.[2,3] Besides this NO mediated signaling also influences solid tumor progression resulting in growth, invasion, metastasis and ability to induce angiogenesis.[4,5] The dual role of NO depends on its threshold level. In normal tissue, NO is generated from L-arginine by Nitric Oxide Synthase (NOS) and the level exceeded the basal level in certain patho-physiological condition and stress condition. This elevated NO level also cause toxicity to the cell and leads into cell death and apoptosis.[6]

The elevated level of NO can be reduced to optimum level either by using NOS inhibitors or NO scavenger. Several arginine analogs have been tested for their NO inhibitory action and as these agents exhibit other effects too, so application becomes limited. Quercetin also screened as a NO scavenger, but threshold maintenance with no or least side effects has not been achieved.[7] Therefore, present study is focused on herbal regime for nitric oxide modulation that might have pharmacological importance.

Phlogacanthus tubiflorus Nees (Family: Acanthaceae) is a traditional medicinal plant used by the tribal population of North Eastern region of India for treating wounds, tumorous growth and also as a blood purifier (Indigenous knowledge). Its bitter

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tested leaves and flowers are used for reliving cough, stomach ache and scabies.[8-10] Flower is also used for treating intestinal worm and rheumatism.[11,12] Leaf extract exhibit antioxidative, acaricidal and fecundity reducing activity.[13,14] Phlocaganthin, a Lacton was isolated from root.[15] The objective of the present study was to investigate the modulatory effects of hydroalcoholic root extracts of *Phlogacanthus tubiflorus* on the Nitric oxide levels in the cells exposed to oxidative stress induced by H_2O_2 and tried to correlate with cell viability.

MATERIALS AND METHODS

Chemical and reagents

Histopaque 1077 and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) obtained from Sigma Chemical Co. (St Louis, MO, USA). RPMI 1640, Fetal Bovine Serum (FBS) were purchased from HiMedia Laboratories (Mumbai, India). The rest of the chemicals were of analytical grade and obtained from local firms of India.

Collection and identification of plant material

The roots of *Phlogacanthus tubiflorus* were collected from Tezpur, Assam (India) and authenticated by a competent Botanist, Prof. S K Borthakur, at the Department of Botany, Gauhati University, Gauhati, Assam (India) and a voucher specimen was preserved in our laboratory. The roots were washed with running tape water repeatedly and finally with distilled water to remove impurities and dried at shade. The dried plant materials were finely powdered and stored in air tight container.

Preparation of plant extract

Dried and coarse powders of *Phlogacanthus tubiflorus* roots (100 g) were macerated with 80% (v/v) ethanol in a shaking condition for one weak. The extract thus obtained (PTE) were filtered concentrated and stored at 4 °C. The extract was dissolved in DMSO with final concentration of 2.5 mg/ml.

Preliminary phytochemical screening

The extract was subjected to phytochemical screening for the detection of polyphenols and flavonoids according to the standard procedure.[16,17]

Isolation and culture of lymphocytes

Lymphocytes were isolated from anticoagulated Chicken blood using Histopaque (1.077 gm/ml) , cultured in RPMI supplemented with 10% heat inactivated fetal bovine serum and were treated with as per experimental requirement. After incubation at 37 °C and 5% CO_2, lymphocytes were centrifuged, washed and homogenized in phosphate buffer saline (PBS). Cell supernatants were used for assaying NO level and protein content.

Determination of nitric oxide levels

NO• levels were determined by the method of Griess in a total volume of 200 µl containing equal volume of Griess reagent and sample and absorbance was read at 550 nm in a microplate reader.[18] The nitrite content in the sample was calculated in mMole NO/mg protein from the standard curve made with sodium nitrite and finally expressed as percentage change of NO level in comparison to control cells.

Cell viability assay

Cell viability was assayed by the method of Denizot and Lang.[19] Briefly, after treatments, cells were treated with 10% of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (Sigma Chem.) for 2 hours and formazan crystals formed were dissolved in solvent as per manufacturer protocol and absorbance was measured at 570 nm. The background absorbance was measured at 690 nm. The absorbance of control cells was set as 100% viable and the values of treated cells were calculated as percentage of control.

Protein determination

The protein contents were determined according to the method described by Lowry et al., (1951) using bovine serum albumin (BSA) as standard.[20]

Statistical Analysis

All the data are expressed as means ± SD, n=3. The significance differences between the experimental and the control groups were analyzed by student's t test and three levels of significance were set as $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS

Preliminary phytochemical screening

Qualitative screening of the plant extract revel the presence of polyphenols and flavonoids in the root extract used for the study.

Effects of different concentrations of H_2O_2

Exposure of lymphocytes to increasing concentration of H_2O_2 (0.1, 0.2, 0.5 and 1%) for 4 hr, significant increase in

the levels of NO was observed. For similar conditions significant decline in the cell viability in concentration dependent manner was observed (Table 1).

Effects of different concentrations of PTE

Lymphocytes treated with 50, 100, 200 and 300 µg/ml of PTE for 4 hr decreases in the level of NO were observed. The decrease in the level of NO was significant at 200 and 300 µg/ml of PTE treatment. In contrast to the NO, the cell viability was found increased in comparison to untreated cells (Table 2).

PTE as modulator in the cells treated with 1% H_2O_2

Lymphocytes were pre-treated with PTE for 1h followed by the treatment with 1% H_2O_2 for 4 h, decline in the level of NO was observed. The cell viability significantly increased for 50, 100 and 200 µg/ml of PTE as compared to positive control (Table 3).

DISCUSSION

It is well known that Nitric oxide (NO), a highly reactive free radical acts as an intracellular messenger and mediate large numbers of signaling pathways. Beside these, NO is also able to react with other inorganic molecules (oxygen, superoxide or transition metals), structures in DNA, prosthetic groups or with the proteins and indirectly causes lipid peroxidation and formation of several harmful products. NO mediated signaling also influences the regulation of apoptosis and cell viability. High level of NO (as in stress condition, inflammatory response) is known to promote apoptotic death and moderate levels of NO exert protective role through inhibition of caspase processing and activation.[21,22] Here, in the present study significant increase in the level of NO was observed in the lymphocytes treated with increasing concentration of H_2O_2 (0.1, 0.2, 0.5 and 1%) for 4 hr and at 1.0% of H_2O_2 treatment NO level increased up to 166.65 % ($p<0.001$) in comparison to untreated cells. This increase in the level of NO might be due to the oxidative stress induced by H_2O_2. For similar conditions significant decline in the cell viability was observed and at 1.0% of H_2O_2 treatment the decline in cell viability was below 50% (Table 1). The results clearly showed the increasing NO level as a result of H_2O_2 treatment induces cell death might be via apoptosis.[6] Treatment of Lymphocyte with different concentration of PTE significantly lowered the NO level (Table 2). In the same treatment condition cell viability increased significantly in comparison to control suggesting protective role of the extract used against endogenous stress. Lymphocytes pre-treated with PTE for 1h, followed by the treatment with 1% H_2O_2 for 4 h, decline in the level of NO was observed and at 100 and 200 µg/ml of PTE treatment, the decline was significant in comparison to only H_2O_2 treated cells. The cell viability significantly increased for 50, 100 and 200 µg/ml of PTE as compared to positive control (Table 3). The results suggest that low level of NO might delay the triggering of apoptosis and hence contributing to increase cell viability.[23] The present data concludes that the NO and cell viability are inter related and root extract of *Phlogacanthus tubiflorus* is rich in phytochemicals like polyphenols, flavonoids and have modulatory effects on the NO level and cell viability and hence, might find pharmacological applications in chemoprevention in future.

Table 1: Effects of different concentrations of H_2O_2. Lymphocytes were treated with PTE for 4 hr.

Condition	% change of NO level (mMole/mg protein)	% change of cell viability
Control	100 ± 7.33	100 ± 5.38
0.1% H_2O_2	112.84 ± 7.92	93.38 ± 6.96
0.2% H_2O_2	118.81 ± 0.57[c]	82.76 ± 7.26[c]
0.5% H_2O_2	123.55 ± 5.43[c]	65.66 ± 5.29[b]
1 % H_2O_2	166.65 ± 10.3[a]	38.18 ± 9.56[a]

Values are mean ± SD; n=3; [a]p<0.001 compared to control cells; [b]p<0.01 compared to control cells; [c]p<0.05 compared to control cells.

Table 2: Effects of the root extract of *Phlogacanthus tubiflorus* (PTE). Lymphocytes were treated with PTE for 4 hr.

Treatments	% change of NO level (mMole/mg protein)	% change of cell viability
Control	100 ± 9.12	100 ± 6.05
50 µg/ml PTE	91.27 ± 5.55	104.63 ± 4.96
100 µg/ml PTE	88.70 ± 0.94	109.19 ± 9.21
200 µg/ml PTE	87.32 ± 1.66	119.64 ± 4.89[c]
300 µg/ml PTE	80.54 ± 2.72[c]	124.11 ± 7.70[c]

Values are mean ± SD; n=3; [a]p<0.001 compared to control cells; [b]p<0.01 compared to control cells; [c]p<0.05 compared to control cells.

Table 3: Protective effects of different concentrations of PTE in H_2O_2 treated lymphocytes. Lymphocytes were pre-treated with PTE for 1 h and followed by 1% H_2O_2 treatment for 4hr.

Treatments	% change of NO level (mMole/mg protein)	% change of cell viability
Control	100 ± 1.96	100 ± 6.29
1% H_2O_2	164.48 ± 7.30[a]	39.45 ± 4.55[a]
1% H_2O_2 + 50 µg/ml PTE	163.74 ± 10.85[a]	71.45 ± 8.80[ce]
1% H_2O_2 + 100 µg/ml PTE	136.03 ± 10.3[bf]	86.14 ± 10.70[e]
1% H_2O_2 + 200 µg/ml PTE	128.36 ± 2.34[ae]	98.33 ± 5.23[d]

Values are mean ± SD; n=3; [a]p<0.001 compared to control cells; [b]p<0.01 compared to control cells; [c]p<0.05 compared to control cells; [d]p<0.001 compared to cells treated with only H_2O_2; [e]p<0.01 compared to cells treated with only H_2O_2; [f]p<0.05 compared to cells treated with only H_2O_2

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ORIGINAL ARTICLE

Oxidative Stress and Modulatory effects of the root extract of *Phlogacanthus tubiflorus* on the activity of Glutathione-S-Transferase in Hydrogen Peroxide treated Lymphocyte

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Glutathione-*S*-transferase is one of the important enzyme systems that plays vital role in decomposition of lipid hydro-peroxides formed due to oxidative stress. In the present study GST activity increased in the lymphocytes treated with increasing concentration of H_2O_2, and decrease in the levels of GSH was observed. For similar treatment conditions LDH activity and MDA levels increased significantly leading to decrease in the cell viability. Treatment of lymphocytes with the root extract of *Phlogacanthus tubiflorus* (PTE) resulted in dose dependent decline in the GST activity and rise in GSH levels. LDH activity and MDA levels also declined that led to the increase of cell viability. Lymphocytes pre-treated with the PTE followed by H_2O_2 (0.1 and 1%) treatment, decline in the activity of GST and increase in GSH levels was observed. Also we have observed decline in the activity of LDH and MDA levels in the lymphocytes for both 0.1 and 1% of H_2O_2 though the magnitude of change was higher in the lymphocytes pre-treated with the PTE followed with 1% of H_2O_2 treatment. Significant increase in the cell viability for similar conditions was also observed. These findings suggest protective function of the root extracts might be through modulation of GST activity and levels of GSH and might find application in Chemomodulation in future.

Key words: Oxidative Stress / Glutathione-S-transferase / Phlogacanthus tubiflorus / Lymphocyte / Chemomodulation.

Abbreviations: ROS: Reactive Oxygen Species, GST: Glutathione-S-transferase, GSH: Reduced Glutathione, LDH: Lactate Dehydrogenase, LP: Lipid Peroxidation, PTE: Phlogacanthus tubiflorus Extract, PBS: Phosphate Buffer Saline.

Oxidative stress causes the production of free radicals and reactive oxygen species (ROS) in the biological system (Subhashinee et al., 2005). The excessive production of such reactive species results in the imbalance in between oxidants and antioxidants that leads to tissue injuries and

contribute to the progression of the several degenerative diseases in humans, such as coronary heart disease, cataracts, muscle degeneration, ageing and cancer (Caporossi et al., 2003; Tandon et al., 2004; Stocker and Keaney, 2004; Shichi, 2004; Ben-Porath and Weinberge, 2005).

Cellular antioxidant enzyme system scavenges and/or neutralizes reactive oxygen species generated under oxidative stress. Glutathione-*S*-transferase (GSTs) (E.C.2.5.1.18) is one of the important constituents of this system, belongs to the super family of enzymes that plays vital role in decomposition of lipid hydro-peroxides formed due to ROS induced peroxidation of the membrane lipids. GST catalyzes the conjugation of reduced glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds, the first step in mercapturic acid pathway that leads to the elimination of toxic compounds (Habing et al., 1974; Hayes et al., 2005).

GSTs have been implicated in the development of resistance towards chemotherapy agents (Hayes and Paulford, 1995; Danyelle and Kenneth, 2003). It is not surprising that levels of GST are elevated in some tumors cells; this may play a role in drug resistance. The elevated levels of GSTs in human tumors can be promising therapeutics targets for research. Numbers of Synthetic compounds and botanicals like phenolics, flavonoids have been studied in in vitro and in vivo systems as a source of antioxidant to modulates the GST activity and subsequent sensitization of tumor cells to chemotherapeutic agents. But, the modulation of GST activity is not enough to combat with detrimental effects of ROS that causes subsequent lowering of drug resistance in chemotherapy (Tew, 1994; Ruzza et al., 2009; Andrea et al., 2010; Elizabeth and Nira, 2010). Also the molecular events associated with modulation of GST activity and other related systems are poorly understood.

The aim of the present study was to investigate the modulatory effects of the root extracts of *Phlogacanthus tubiflorus* (Family: Acanthaceae) on the activity of the glutathione-*S*-transferase in the lymphocytes, cultured *in vitro*, and exposed to hydrogen peroxide (H_2O_2). The selection of the plant is based on the ethno-pharmacological data obtained by consulting the traditional healer and thereafter consulting the scientific literature related to the identified plant. *Phlogacanthus tubiflorus Nees* is traditionally used by the tribal population of North Eastern Region of India for treating wounds, tumorous growth and also as a blood purifier (Indigenous knowledge). Besides this we have also investigated the levels of reduced glutathione (GSH), lipid peroxidation (LP) and Lactate dehydrogenase (LDH, EC 1.1.1.27) activity and tried to correlate with the GST activity.

MATERIALS AND METHODS

Preparation of Modulator

The roots of *Phlogacanthus tubiflorus* were collected from Tezpur, Assam (India) and were authenticated by a competent Botanist, Prof. S K Borthakur, at the Department of Botany, Gauhati University, Gauhati, Assam (India) and Voucher specimen was preserved in our laboratory. The roots were washed with running tape water repeatedly and finally with distilled water to remove impurities and dried at shade. The dried plant materials were finely powdered and macerated with 80% (v/v) ethanol in a shaking condition. The extract thus obtained (PTE) were filtered and concentrated and stored at 4^0 C. The extract was dissolved in DMSO with final concentration of 2.5 mg/ml.

Isolation of Lymphocytes

Chicken blood was collected from source and was diluted 1:1 with PBS then layered 6 ml into 6 ml Histopaque (1.077 gm/ml). Lymphocytes were isolated from the sample after centrifugation for 30 minutes at 400 g. Lymphocytes were then washed with 2 ml PBS and 2 ml serum free media separately through centrifugation for 10 minutes at 250 g. Cell pellets were then suspended in PBS and cell viability was checked by Trypan blue exclusion method using haemocytometer. Cell viability more than 90 % was used for subsequent study.

Lymphocytes culture and treatment

Aliquots of 200 µl of isolated lymphocytes were seeded in 96 well culture plate in RPMI supplemented with 10% heat inactivated Fetal bovine serum (FBS) and were treated with H_2O_2 / PTE / PTE+H_2O_2 as per experimental requirements and maintained at 37^0C and 5 % CO_2 in CO_2 incubator. Lymphocytes were treated for 4 hours in case of only H_2O_2 and PTE treatment while in other cases pre treated with PTE for 1 hour and then treated with H_2O_2 for 4 hours. After incubation, lymphocytes were centrifuged and washed with PBS, homogenized in PBS. Cell supernatants were used for assaying GST, GSH, Protein and LP while cell free media were used for assaying LDH.

Glutathione-*S*-Transferase

The specific activity of cytosolic GST was determined spectrophotometrically (Habig et al., 1974). In brief, the reaction volume (1 ml) contained final concentration of 0.1 M phosphate buffer (pH 6.5), 1 mM CDNB in 95% ethanol and 1 mM GSH and was incubated at 37^0C for 5 min. The reaction was initiated by the addition of enzyme sample and the activity was measured for 3 min at 340 nm (Cecil Aquarius, 7000 series). The specific activity of GST was calculated using the extinction coefficient 9.6 $mM^{-1}cm^{-1}$ at 340 nm and expressed in terms of percentage change of µ mole of CDNB-GSH conjugates formed/min/mg proteins.

Reduced glutathione

Reduced glutathione content was estimated as the total non-protein sulphydryl group by the standard procedures (Moron et al., 1979). The proteins were precipitated by addition of tricholoroacetic acid (TCA), centrifuged and supernatant was collected. The supernatant was mixed with 0.2 M phosphate buffer (pH 8) and 0.6 M 5,5'-dithio-bis (2-nitrobenzoic acid) dissolved in 0.2 M phosphate buffer, and allowed to stand for 8-10 min at room temperature. The absorbance was recorded at 412 nm using a spectrophotometer (Thermo Scientific, UV 10). Reduced glutathione (GSH) was used as a standard to calculate nMole of –SH content/mg protein and finally expressed as percentage change of GSH level.

Lactate Dehydrogenase

The specific activity of Lactate dehydrogenase (LDH) released into the medium as a result of membrane damage was assayed by measuring the rate of oxidation of NADH at 340 nm (Bergmeyer and Bernt, 1974). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.5), 0.5 mM sodium pyruvate, 0.1 mM NADH and required amount cell free media to make the final volume of 1 ml. The reaction was started at 25^0C by addition of NADH and the rate of oxidation of NADH was measured at 340 nm using a spectrophometer (Cecil Aquarius, 7000 series). The enzyme activity was calculated using extinction coefficient 6.22 $mM^{-1}Cm^{-1}$/mg protein and finally expressed as percentage change of LDH activity.

Lipid Peroxidation

Peroxidative damage was estimated spectrophotometrically by the assay of

thiobarbituric acid reactive substances (TBARS) and expressed in terms of nMole of malondoialdehyde (MDA) formed per mg protein (Okhawa et al., 1979). Briefly, in a 3 ml reaction volume cell homogenate supernatant was mixed with 0.15 M Tris–KCl buffer (pH 7.4) and 30% tricholoroacetic (TCA) and 52 mM thiobarbituric acid (TBA). The mixture was heated for 45 minutes at 80^0 C, cooled and centrifuged for 10 minutes at 3000 rpm. The absorbance of the clear supernatant was measured against distilled water blank at 531.8 nm in spectrophotometer (Thermo Scientific, UV 10) and finally expressed as percentage change of nMole of MDA formed per mg protein.

Cell Viability assay

Cell viability assay were performed according to the MTT based method (Denizot and Lang (1986). The key component (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT) is yellowish in color and mitochondrial dehydrogenase of viable cells cleave the tetrazolium ring, yielding purple insoluble formazan crystals which were dissolved in suitable solvent. The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material. Briefly, after treatments, lymphocytes were treated with 10 % of MTT for 2 hours and after which formazan crystals were dissolved in solvent and its absorbance were measured at 570 nm. The absorbance of control cells was set as 100% viability and the values of treated cells were calculated as percentage of control.

Protein determination

The protein contents were determined using bovine serum albumin (BSA) as standard (Lowry et al., 1951).

Statistical Analysis

All the data are expressed as means ± SD, n=3. Results were statistically analyzed by student's t test for significance difference between group mean using GraphPad software (Bourke et al., 1985). The significance difference between the experimental and the control group was set at different levels as $p< 0.05$, $p< 0.01$ and $p< 0.001$.

RESULTS

Effects of different concentrations of H_2O_2 (Table 1)

Exposure of lymphocytes to increasing concentration of H_2O_2 (0.1, 0.2, 0.5 and 1%), increase in the activity of GST in dose dependent manner was observed and at 1.0% H_2O_2 treatment GST activity increased to 168.42% ($p<0.01$) in comparison to untreated cells. For similar conditions significant decline in the levels of GSH was observed. Per-oxidation of membrane lipids is indicator of oxidative stress experienced by the cells. Dose dependent increase in the level of lipid peroxidation was observed on treatment with H_2O_2. The increase in the lipid peroxidation was significant for all the dose of H_2O_2 used in the experiment. Further we also observed increase in LDH activity and decline in the cell viability. For 1.0% of H_2O_2 treatment the decline in cell viability was below 50% (Figure 1a).

Effects of the root extract of *Phlogacanthus tubiflorus* (PTE) (Table 2)

Lymphocytes treated with 50, 100 and 200 µg/ml of root extracts of *Phlogacanthus tubiflorus* decrease in the activity of GST was observed. The decrease in the GST activity was significant at 100 and 200 µg/ml of root extracts used in the experiment. In contrast to the GST activity,

significant increase in the levels of GSH was observed. The treatment of the root extracts resulted in the dose dependent decline in the levels and activity of MDA and LDH respectively. For the similar conditions the cell viability also increased in comparison to untreated cells (Figure 1b).

PTE as modulator in the cells treated with 0.1% H_2O_2 treatment

Dose dependent increase in the levels of reduced Glutathione (GSH) was observed in the lymphocytes pre-treated with root extracts followed by 0.1% H_2O_2 treatment for 4 h. For similar conditions decline in the activity of GST was observed. Significant decline in the levels of MDA and activity of LDH respectively were observed. Cell viability increased for all doses of the root extracts used in experiment (Figure 1c).

Root extracts of PTE as modulator in the cells treated with 1% H_2O_2

Lymphocytes were pre-treated with root extracts of PTE for 1h followed by the treatment with 1% H_2O_2 for 4 h, decline in the activity of GST was observed. Glutathione levels increased in dose dependent manner as compared to positive control. For similar conditions decline the levels and activity of MDA and LDH was observed as compared to the positive control but the overall activity and levels were significantly high as compared to the negative control. The cell viability increased for 50, 100 and 200 µg/ml of the root extract used as compared to positive control (Figure 1d).

Table 1: Effects of different concentrations of H_2O_2. Lymphocytes were treated with the indicated concentrations of H_2O_2 for 4h and activity of GST and LDH; and level of GSH and MDA were measured as described in material and method section. Values are mean ± SD; n=3; $^{a}p<0.001$ compared to control cells; $^{b}p<0.01$ compared to control cells; $^{c}p<0.05$ compared to control cells.

Treatments (H_2O_2)	% Change of GST activity (unit/mg protein)	% Change of GSH level (nMole/mg protein)	% Change of LDH activity (unit/mg protein)	% Change of MDA level (nMole/mg protein)
Control	100±7.49	100±8.83	100±7.45	100±4.76
0.10%	108.76±7.25	92.75±4.77	155.79±10.39 ^{b}	110.69±2.79
0.20%	120.97±1.12 ^{b}	86.40±0.64	172.34±11.49 ^{a}	139.63±9.31 ^{b}
0.50%	133.54±8.90 ^{b}	67.05±4.47 ^{b}	217.22±14.48 ^{a}	202.01±2.03 ^{a}
1%	168.42±6.74 ^{b}	45.31±2.79 ^{a}	264.32±17.62 ^{a}	251.55±16.77 ^{a}

Table 2: Effects of the root extracts of *Phlogacanthus tubiflorus* (PTE). Lymphocytes were treated with the indicated concentrations of PTE for 4h and activity of GST and LDH; and level of GSH and MDA were measured as described in material and method section. Values are mean ± SD; n=3; $^{a}p<0.001$ compared to control cells; $^{b}p<0.01$ compared to control cells; $^{c}p<0.05$ compared to control cells.

Treatments (PTE)	% Change of GST activity (unit/mg protein)	% Change of GSH level (nMole/mg protein)	% Change of LDH activity (unit/mg protein)	% Change of MDA level (nMole/mg protein)
Control	100±6.67	100±8.12	100±2.67	100±1.99
50µg/ml	88.04±5.87	119.53±7.97 ^{c}	91.35±6.09	98.52±6.57
100 µg/ml	80.94±5.40 ^{c}	136.68±9.11 ^{b}	66.22±4.42 ^{a}	91.89±6.13
200 µg/ml	72.53±4.84 ^{b}	139.71±9.31 ^{b}	63.15±4.21 ^{a}	76.85±5.12 ^{b}

Table 3: Protective effects of different concentrations of PTE in H_2O_2 treated lymphocytes. Lymphocytes were pre-treated with PTE for 1 h and followed by 0.1% H_2O_2 treatment for 4h and activity of GST and LDH; and level of GSH and MDA were measured as described in material and method section. Values are mean ± SD; n=3; [a]p<0.001 compared to control cells; [b]p<0.01 compared to control cells; [c]p<0.05 compared to control cells; [d]p<0.001 compared to cells treated with only H_2O_2; [e]p<0.01 compared to cells treated with only H_2O_2; [f]p<0.05 compared to cells treated with only H_2O_2.

Treatments	% Change of GST activity (unit/mg protein)	% Change of GSH level (nMole/mg protein)	% Change of LDH activity (unit/mg protein)	% Change of MDA level (nMole/mg protein)
Control	100±6.67	100±6.67	100±6.67	100±6.67
H_2O_2 (0.1%)	111.97±7.46	93.73±6.25	148.00±9.87 [b]	121.34±8.09 [c]
H_2O_2 (0.1%) + PTE(50 µg/ml)	74.55±5.22 [be]	112.53±7.50	132.26±8.82 [b]	115.24±7.68
H_2O_2 (0.1%) + PTE(100 µg/ml)	67.42±4.49 [bd]	127.86±8.52 [ce]	96.26±8.82 [e]	96.88±4.42 [f]
H_2O_2 (0.1%) + PTE(200 µg/ml)	70.85±4.72 [be]	140.57±9.37 [be]	44.70±2.98 [ad]	66.80±4.42 [bd]

Table 4: Protective effects of different concentrations of PTE in H_2O_2 treated lymphocytes. Lymphocytes were pre-treated with PTE for 1 h and followed by 1% H_2O_2 treatment for 4h and and activity of GST and LDH; and level of GSH and MDA were measured as described in material and method section. Values are mean ± SD; n=3; [a]p<0.001 compared to control cells; [b]p<0.01 compared to control cells; [c]p<0.05 compared to control cells; [d]p<0.001 compared to cells treated with only H_2O_2; [e]p<0.01 compared to cells treated with only H_2O_2; [f]p<0.05 compared to cells treated with only H_2O_2.

Treatments	% Change of GST activity (unit/mg protein)	% Change of GSH level (nMole/mg protein)	% Change of LDH activity (unit/mg protein)	% Change of MDA (nMole/mg protein)
Control	100±6.67	100±8.48	100±5.62	100±6.32
H_2O_2 (1%)	143.26±9.55 [b]	46.16±3.08 [a]	280.93±9.87 [a]	257.70±4.16 [a]
H_2O_2 (1%) + PTE(50 µg/ml)	80.40±5.36 [cd]	90.32±6.19 [d]	250.51±16.70 [a]	219.13±14.61 [a]
H_2O_2 (1%) + PTE(100 µg/ml)	72.60±4.84 [bd]	118.66±7.91 [cd]	161.52±10.77 [ad]	212.75±14.18 [ae]
H_2O_2 (1%) + PTE(200 µg/ml)	79.92±5.33 [cd]	142.48±9.50 [bd]	123.04±13.52 [d]	196.5±13.09 [ae]

DISCUSSION

Here in the present study dose dependent increase in the GST activity and decline in the levels of glutathione was found in lymphocytes treated with increasing concentration of H_2O_2. GSTs are present in many organs and have been implicated in the detoxification of endogenous α,β unsaturated aldehydes formed during lipid peroxidation induced by oxidative damage (Esterbaucer et al.,1991) . The increase in the GST activity is due to the formation of lipid peroxides in the cells on H_2O_2 treatment. This is indicated by increase in the levels of MDA formation in the cell (Milei et al., 2007) (Table 1). GSH is co factor of GST and is responsible for the redox status of cell. The decline in the levels of GSH is due to increase in the activity of GST. The significant increase in the levels of lipid peroxides

measured as MDA formation has led to significant fall in the cell viability suggesting the cellular damage at all concentration of H_2O_2 treatment. This is also indicated by the increase in the activity of LDH released in to the media (Goswami et al., 2003; Kim et al., 2008).

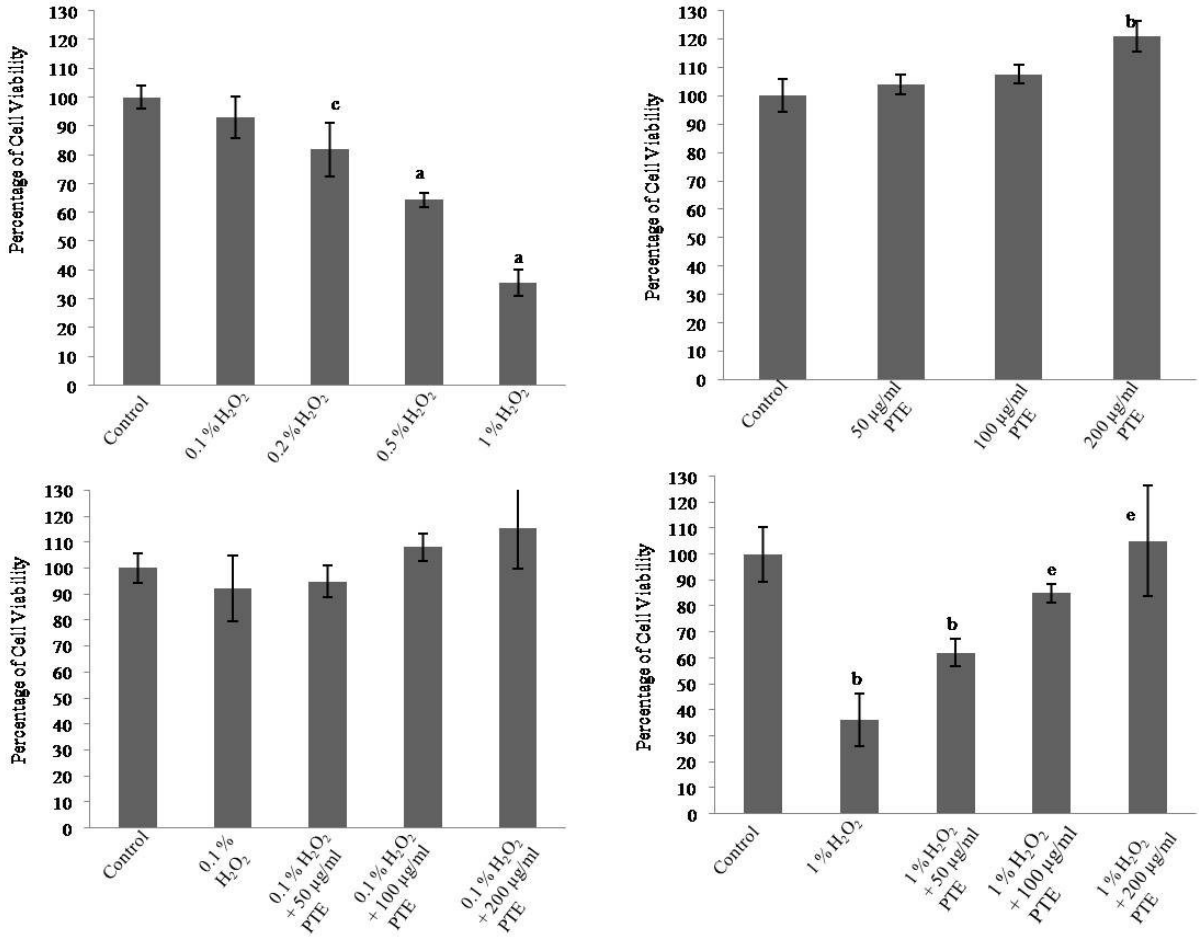

Figure 1: Results Cell Viability measured by MTT Assay. (a) Effects of different concentration of H_2O_2; (b) Effects of different concentration of PTE; (c) Pre-exposure of cells with PTE followed by 0.1 % H_2O_2; (d) Pre-exposure of cells with PTE followed by 1% H_2O_2 treatment. Values are mean ± SD; n=3; $^{a}p<0.001$ compared to control cells; $^{b}p<0.01$ compared to control cells; $^{c}p<0.05$ compared to control cells; $^{d}p<0.001$ compared to cells treated with only H_2O_2; $^{e}p<0.01$ compared to cells treated with only H_2O_2; $^{f}p<0.05$ compared to cells treated with only H_2O_2.

When the lymphocytes were treated with root extracts of *Phlogacanthus tubiflorus*, decline in the activity of GST and increase in the levels of GSH was observed. As GST is involved in the detoxification of lipid peroxides, significant fall in the levels of lipid peroxidation might have led to decline in the activity of GST. In contrast the cell viability increased significantly with the treatment of the root extracts (figure 1b), this is indicated from the decline in the LDH activity for similar conditions. This finding suggests the antioxidant property of root extracts as the treatment has led to the dose dependent decrease in the lipid peroxide levels and significant modulation of GSH levels and GST activity (Saravanan et al., 2003; Dahiru et al., 2005; Pardhasarathi et. al., 2005). This might have resulted in the significant increase in the cell viability.

In the lymphocytes pre-treated with the root extracts 50, 100 and 200 µg/ml for 1h and followed by the 0.1% H_2O_2 treatment for 4h, decline in the activity of GST was observed. This decline in the activity of GST might be due to decreased levels of lipid peroxides formed for similar conditions as indicated by level of MDA formation (Table 3). As GSH is one of the co factors, decline in the activity of GST resulted in the increase GSH levels in the cells. For the similar conditions significant increase in the cell viability was observed. The decline in activity of LDH suggests reduced levels of oxidative stress experience by cells in comparison to the positive control (Rouach et al., 1997; Ramteke et al., 2007).

When the lymphocytes were pre-treated with the root extracts for 1h and followed by the treatment of 1% H_2O_2 for 4 h, decrease in the activity GST was found. As expected GSH levels increased and this increase was highly significant in comparison to the positive control at higher concentration. This might have results 200 fold increased in the cell viability up to 100 µg/ml of root extract treatment and thereafter at 200 µg/ml it declined. This decline in the cell viability at higher concentration of root extracts treatment might be due to the higher levels lipid peroxides and LDH activity. This suggests the synergistic action of the higher concentrations of root extract with the 1% H_2O_2 that have resulted in the decline in the cell viability. The exact reason for this finding is not clear from the present findings and needs further investigations (Parraga et al*.,* 2003; Dash et al., 2008).

From the present finding it could be concluded the root extracts of *Phlogacanthus tubiflorus* is rich in active principles with antioxidant properties and that might be responsible for the protective role against oxidative stress induced by H_2O_2 in the lymphocytes. GST and Glutathione have been implicated in the development of resistance towards chemotherapeutic agents. The therapeutic applications of the root extract of *Phlogacanthus tubiflorus* as modulators of GST and Glutathione needs further investigations in mammalian system.

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