# International Journal of Botany

ISSN: 1811-9700

science alert

ANSInet
an open access publisher
http://ansinet.com

International Journal of Botany 11 (1): 21-26, 2015
ISSN 1811-9700


RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ijb.2015.21.26

# Inhibitory Potential of *Terminalia chebula* by Hydrogen Peroxide Induced Oxidative Stress in THP-1 Cell Line

[1]G.C. Minakshi, [1]K. Vasanth, [1]Tanupriya, [1]K. Ilango, [2]Aruna Agrawal and [3]G.P. Dubey
[1]Interdisciplinary Institute of Indian System of Medicine (IIISM), SRM University, Kattankulathur, 603203, Kancheepuram (Dt), Tamil Nadu, India
[2]Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221 005, Uttar Pradesh, India
[3]National Facility for Tribal and Herbal Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005, Uttar Pradesh, India

ARTICLE INFO
*Article History:*
Received: June 11, 2015
Accepted: July 23, 2015

Corresponding Authors:
G.C. Minakshi
Interdisciplinary Institute of Indian System of Medicine (IIISM), SRM University, Kattankulathur, 603203, Kancheepuram (Dt), Tamil Nadu, India
G.P. Dubey
National Facility for Tribal and Herbal Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005, Uttar Pradesh, India
Tel: +91 44 47432341
Fax: +91 44 47432342

ABSTRACT
Diseases caused due to 'oxidative stress' initiated by Reactive Oxygen Species (ROS) is growing at an alarming rate. Several medicinal herbs explored till date were reported to possess numerous pharmacological properties used for combating different diseases. The present study was undertaken to investigate the inhibitory potential of *Terminalia chebula* by $H_2O_2$ induced oxidative stress in THP-1 cell line and also the presence of the marker compounds in *T. chebula* was confirmed by High Performance Liquid Chromatography (HPLC). Intracellular quantification of ROS was done using a flow cytometer. The percentage of gallic acid and ellagic acid in the extract was performed by HPLC were found to be 4.84% w/w and 2.56% w/w, respectively. Pretreatment of cells with *T. chebula* extract significantly reduced the intracellular ROS production in a dose-dependent manner. The present study illustrates the effective role of *T. chebula* in reducing the ROS production which is a causative factor for several disorders. The results describe the possibility and potentiality of natural sources to inhibit the pathogenesis of different diseases caused due to oxidative stress.

**Key words:** Oxidative stress, *Terminalia chebula*, antioxidants, reactive oxygen species

## INTRODUCTION

During the last few years, new and promising advances have been made by scientists to explore various traditional medicines. The western medicines can cause adverse effects and has been known to develop intolerance in patients towards its pharmacotherapy (Sham *et al*., 2014). Therefore herbal medicines are preferred over synthetic ones due to their natural occurrence, presumed safety, nutritional and therapeutic potential. Several herbal medicines as well as purified natural products provide a rich resource for development of many novel drugs (Lin *et al*., 2014). One among the traditional folk medicines *Terminalia chebula*, belonging to the family Combretaceae has been used extensively in traditional as well as the modern practice against diabetes, cancer and bacterial infections (Lee *et al*., 2015). The plant has also been known to possess several pharmacological properties such as antioxidant, anti-inflammatory, antimutagenic, cardioprotective and wound healing activity (Bag *et al*., 2013). Though different parts of this plant have been explored for various pharmacological properties (Maddinedi *et al*., 2015; Kathirvel and Sujatha, 2012), the fruits have been known to exhibit several compounds such as hydrolysable tannins, anthraquinone, flavanol and terpenoids and reported for several therapeutic properties like anti-diabetic, anti-mutagenic confirmed by several *in vitro* and *in vivo* studies (Rekha *et al*., 2014). It also demonstrated to be a promising source of antioxidants (Mathew and Subramanian,

2014). The present study utilizes the dried fruit extract of *T. chebula* as it was observed to have a good efficacy in radical scavenging activity (Saha and Verma, 2014).

The diseases caused due to 'oxidative stress' which is initiated by Reactive Oxygen Species (ROS), produced as a byproduct of the electron transport in mitochondria is growing at an alarming rate (Saha and Verma, 2014). The ROS is considered to be an important target in the development of several anti-cancer drugs. Harmful free radicals like hydroxyl and superoxide anions are being constantly produced as a result of the metabolic reactions occurring inside the living system. The inhaled oxygen may be converted to ROS that can bind to cellular structures and is involved in a number of pathophysiological processes such as aging, inflammation and atherosclerosis (Singhal *et al*., 2014). Excessive accumulation of ROS can lead to protein oxidation, lipid peroxidation, enzyme inactivation and oxidative DNA damage (Lv *et al*., 2013). Keeping in view the use of *T. chebula*, as an effective remedy for oxidative stress associated diseases, the present study was undertaken to evaluate the inhibitory potential of *T. chebula* fruit extract by hydrogen peroxide ($H_2O_2$) induced ROS generation in THP-1 cell line.

## MATERIALS AND METHODS

**Plant identification and extract preparation:** *Terminalia chebula* was collected from the natural habitat of Salem district, Tamil Nadu, India, in December 2012. The samples were identified and authentication was done by a plant anatomist. A Voucher specimen (ISISM/RES/B0014) has been preserved at the department of the Interdisciplinary Institute of Indian System of Medicine (IIISM), SRM University for future reference. The plant materials were shade dried at room temperature, powdered and stored in air tight containers. Thirty grams of the powdered plant material were soaked in a hydro-alcoholic mixture at room temperature with continuous shaking and the extraction was repeated three times. After completion of the extraction process, the solvent was removed using a rotary vacuum evaporator (Rotovac R-210, Buchi, Germany). The extract was stored in an air-tight container for further use.

**Identification and quantification of active compounds by HPLC:** *Terminalia chebula* extract was dissolved in HPLC grade MeOH (1 mg $mL^{-1}$) and subjected to Reverse Phase High Performance Liquid Chromatography (RP-HPLC) for the qualitative and quantitative analysis of gallic acid and ellagic acid content. The HPLC system consisted of Shimadzu LC 2020 (Shimadzu, Japan) equipped with solvent delivery system, column oven, Photo Diode Array detector (PDA) and Lab solution software was used for all data analysis. The chromatographic separation was performed on Phenomenex-Luna (Torrance, CA, USA) $C_{18}$ column (i.d. 250×4.6 mm, 5 μm) and the column oven were set at ambient temperature. The isocratic mobile phase consisted of methanol: Acetonitrile: Water (0.05% formic acid, 40:15:45) with a flow rate of 0.8 mL $min^{-1}$ and the samples were injected using Hamilton Micro liter syringe (Switzerland) into a 20 μL injection loop.

**Cell line and treatment:** Human monocytic (THP-1) cell line were purchased from National Center for Cell Sciences, Pune, India. The cells were cultured under standard conditions in the roswell park memorial institute (RPMI-1640) supplemented with 10% Fetal bovine serum (FBS), 100 U $mL^{-1}$ of penicillin and 100 U $mL^{-1}$ of streptomycin, HiMedia, India in a humidified 5% $CO_2$ at 37°C.

**Cytotoxicity assay:** The cytotoxic effect of *T. chebula* on THP-1 cells was assessed by measuring the activity of mitochondrial dehydrogenase as described previously (Wang *et al*., 2010). Briefly cells ($5\times10^3$ cells/well) were incubated with various concentrations (3.9-500 μg $mL^{-1}$) of *T. chebula*, the culture media was removed and the cells were incubated with 5 mg $mL^{-1}$ 3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyltetrazolium bromide, Sigma Aldrich, India) (MTT) in fresh medium at 37°C for an additional 4 h. After this period, the supernatant was removed and 100 μL dimethyl sulfoxide, Sigma Aldrich, India, (DMSO) was added to each well to dissolve the formazan crystals. The plates were read on a microplate reader using MultiSkan GO, Thermo Scientific, USA, at a test wavelength of 540 nm with a reference wavelength of 650 nm.

**Quantification of intracellular ROS in $H_2O_2$ induced THP-1 cells:** The ROS generation was measured after staining the cells with oxidation sensitive probe 2-7-diacetyl dichlorofluorescein, Invitrogen, USA, (DCFH-DA) using flow cytometry as described previously with minor modifications (Krejsa and Schieven, 2000; Vasanth *et al*., 2014). THP-1 cells ($2\times10^5$ cells/well) were cultured in 12-well plate treated with indicated concentrations in the presence or absence of *T. chebula* fruits extract (62.5, 125 and 250 μg $mL^{-1}$) and incubated at 37°C with 5% $CO_2$ for 24 h. The cells in 12-well plates were washed twice with Hank's Balanced Salt Solution (HBSS) and incubated in 1 mL working solution of 20 μM DCFH-DA at 37°C in the dark for 30 min. Then the cells were harvested, washed with ice-cold Phosphate Buffered Saline (PBS). Cells were treated with 10 mM $H_2O_2$ to induce intracellular ROS. The fluorescence emission of DCF were analyzed in FL-1 channel on Fluorescence Activated Cell Sorting (FACS) (JazzBecton Dickinson, USA) using excitation and emission wavelengths at 488 and 525 nm, respectively. The Mean Fluorescence Intensity (MFI) of $10^4$ cells acquired were quantified.

**Statistical analysis:** Data are expressed as Mean±SD. The statistical significance of the differences between the mean of

various experimental groups was determined by one-way ANOVA followed by Bonferroni test using prism program (Graph-Pad Software Inc., USA). The level of the statistical significance was set at $p<0.05$.

## RESULTS

**HPLC analysis:** The HPLC have been utilized to standardize many medicinal plant extracts. Standardization of plant based products is more challenging than synthetic drugs. In the present study, *T. chebula* fruit extract was subjected to HPLC analysis to find out the presence of biomarkers which could be due its synergistic pharmacological activities. The HPLC chromatogram of reference standards like gallic acid and ellagic acid were detected at 280 and 254 nm, respectively is showed Fig. 1. The presence of gallic acid and ellagic acid, was confirmed by comparing chromatographic peaks in hydro-alcoholic extract of *T. chebula* fruits with the Retention Time (Rt) 3.5 and 5.79 min, respectively. Compounds are reputed for their health promoting properties due to their high antioxidant capacity. The percentage of gallic acid in the *T. chebula* extract is 4.84% w/w and that of ellagic acid is 2.56% w/w.

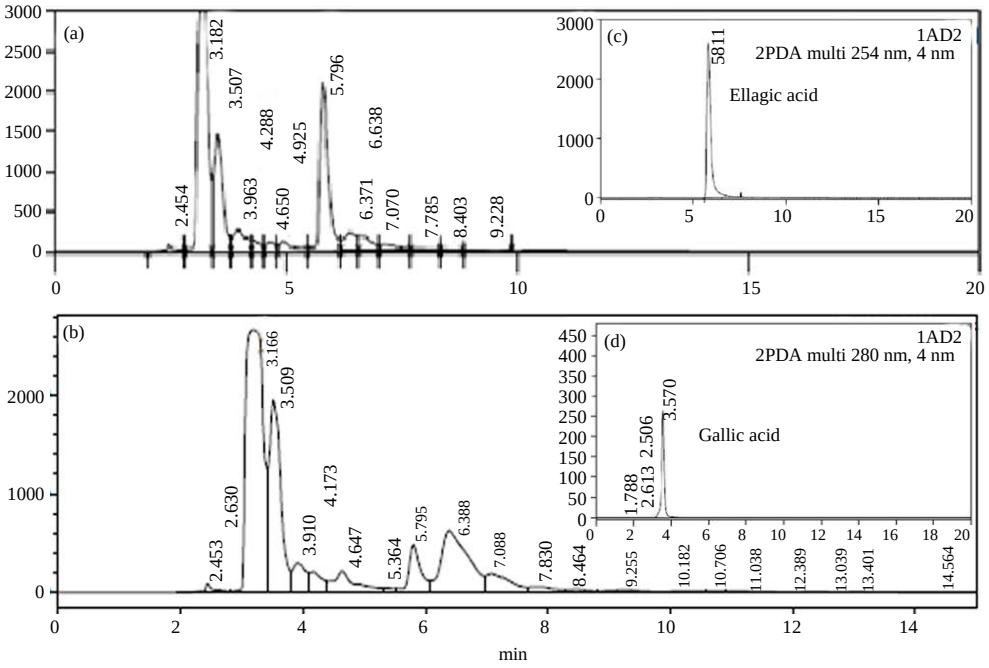


Fig. 1(a-d): HPLC chromatogram representing (a, b) Hydroalcoholic extract of *Terminalia chebula* fruits at 254 and 280 nm, respectively inset, (c) Standard ellagic acid and (d) Standard gallic acid

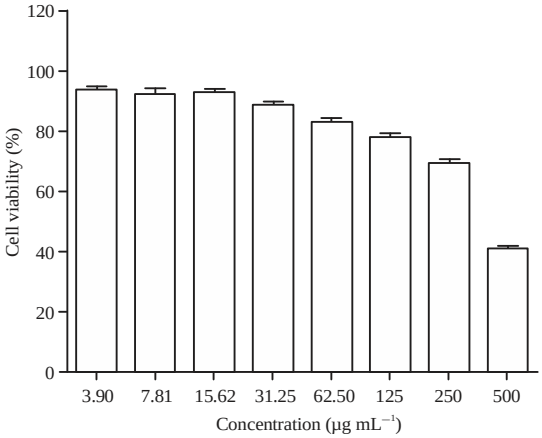


Fig. 2: Effect of *Terminalia chebula* fruit extract on cellular viability of THP-1 cells was examined by the MTT assay

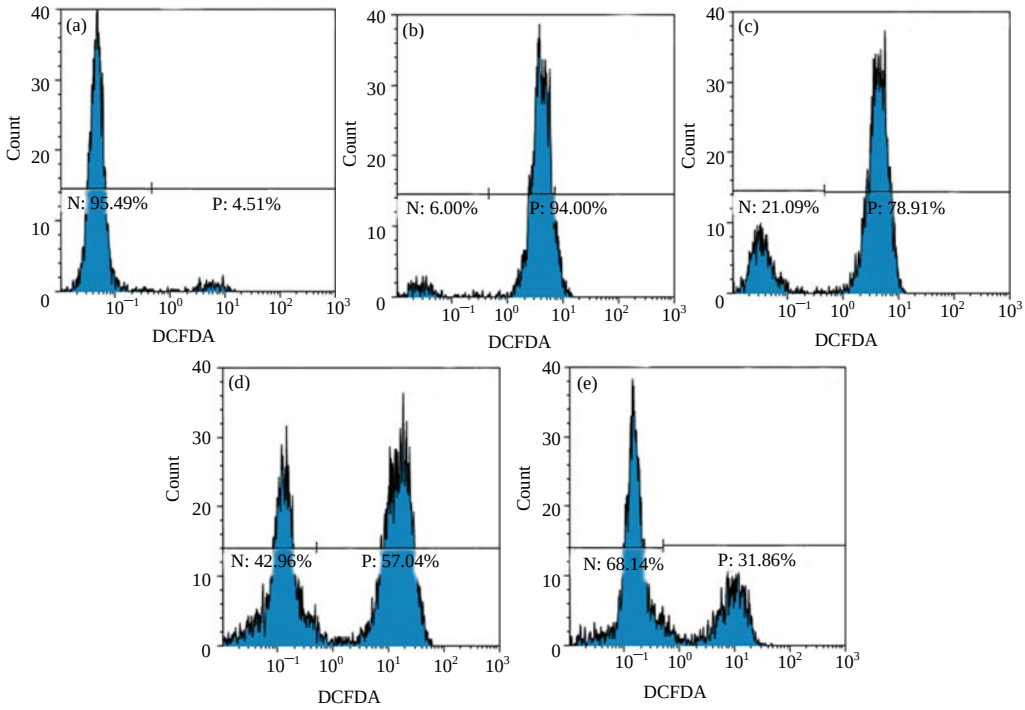


Fig. 3(a-e): Effect of *Terminalia chebula* fruit extract on the $H_2O_2$ induced ROS production in THP-1 cells. Cells were stained with DCFH-DA and analyzed using FACSCalibur flow cytometer, (a) Control, (b) $H_2O_2$, (c) $H_20_2$+62.5, (d) $H_2O_2$+125 and (e) $H_2O_2$+250 μg $mL^{-1}$

**Effect of *Terminalia chebula* on cell viability:** The MTT assay was performed to determine the safe concentration range of *T. chebula* in THP-1 cell. As shown in Fig. 2, the HA extract of *T. chebula* did not exhibit cytotoxicity to THP-1 cells at doses of 3.9-500 μg $mL^{-1}$. However, the extract inhibited cell viability at the highest dose that was tested (500 μg $mL^{-1}$). Thus, doses of *T. chebula* at the concentration that gave cell viability of 80% or more (62.5, 125 and 250 μg $mL^{-1}$ were used for treatment in the further experiments. These results indicate that the *T. chebula* did not induce cell death.

**Effect of intracellular ROS in $H_2O_2$ induced THP-1 cells:** Flow cytometric analysis of THP-1 cells treated with different concentrations (62.5, 125 and 250 μg $mL^{-1}$) of *T. chebula* stained with DCFH-DA, an indirect method for estimating the intracellular ROS, a specific fluorescent probe for measuring hydrogen peroxide and hydroxyl radicals revealed an increase in DCFH-DA positive cell population indicating generation of reactive oxygen species. As shown in Fig. 3, FACS analysis revealed that exposure to $H_2O_2$, markedly increased intracellular ROS levels in THP-1 cells, compared with the control group. Whereas, pretreatment of cells with *T. chebula* extract significantly reduced the intracellular ROS production in a dose-dependent manner.

## DISCUSSION

The present study was undertaken to evaluate the inhibitory potential of *T. chebula* by hydrogen peroxide induced ROS generation in THP-1 cell line. It is a human leukemia monocytic cell line considered as a suitable, reliable model to study the functions and responses of monocytes and macrophages (Chanput *et al*., 2014). Herbal drugs have been used since traditional times in the treatment of various diseases. Antioxidants as external supplements are recommended to protect cells from the deleterious effects of oxidative stress conditions. In search of novel and natural antioxidants, various herbal extracts have been explored and researched in the preparation of Ayurvedic formulations. A robust investigation was done on 75 plants for their *in vivo* anti-inflammatory properties (Kam *et al*., 2014). Continuous research on the molecular mechanism of the diseases caused due to oxidative stress has resulted in identification of important targets necessary for drug development and thus allowed the investigation of various plant extracts in different cell based assays. One amongst the most popularly used plants since ancient times is *T. chebula* known for several decades for its various pharmacological properties (Bag *et al*., 2013). The fruits of this plant have been known to exhibit several medicinal properties such as anti-diarrheic, styptic, anti-bilious and anti-dysenteric activities as been reported by Cheng *et al.* (2003). The different pharmacological properties of compounds isolated from the fruits of this plant have been reported (Chen *et al*., 2011). For example chebulagic acid showed anti-proliferative and pro-apoptotic activities in several tumor cell lines. Arjunic acid isolated from this plant was also known as a potent free radical scavenger.

The role of ROS has been implicated in the pathogenesis of many chronic diseases like cancer. It has been known to affect the mitochondrial membrane potential and membrane permeability that can stimulate a number of mitochondria related events. Excessive accumulation of ROS can lead to oxidative DNA damage, protein oxidation and enzyme inactivation. This proves its usefulness in the development of several anti-cancer drugs (Lv *et al*., 2013). An imbalance of the ROS generation can aggravate inflammation. In the past few years the participation of ROS in the pathogenesis of several diseases has been given several importance. ROS such as hydrogen peroxide, superoxide anions and hydroxyl radicals can cause damage to tissues by activation of the NF-κB pathway and thus initiate as well as perpetuate the inflammatory cascade (Gusman *et al*., 2015).

Two important biomarker compounds were identified from this plant using HPLC. Gallic acid, a phenolic compound present in *T. chebula* has been reported for several health benefits such as anti-cancer, anti-inflammatory and cardioprotective properties (Kam *et al*., 2014). It demonstrated potent antioxidant properties which neutralizes ROS generation and oxidative stress and known to inhibit the tumor growth of liver and colon (Subramanian *et al*., 2014). Another biomarker, ellagic acid has gained much importance in the recent years due to its multiple pharmacological activities and molecular targets. It has shown several antibacterial, anticarcinogenic and antiatherogenic activities (Garcia-Nino and Zazueta, 2015). Several studies have enumerated the role of ellagic acid in effective lowering of plasma lipids, reduced oxidative stress, inhibition of apoptosis, scavengers of oxygen and hydroxyl radicals as well as inhibition of lipid peroxidation both *in vitro* and *in vivo*. It has been known to inhibit the cytokine induced ROS generation and expression of adhesion molecules as well as prevention of oxidized low Density Lipoprotein (LDL) induced apoptosis in the endothelial cells (Lee *et al*., 2010). The role of *T. chebula* in inhibiting ROS production may be attributed due to the symbiotic effect of these two important marker compounds present in the extract.

## CONCLUSION

*Terminalia chebula* suppressed the production of ROS, known to participate in the causation of several diseases. It exhibited a high potential against markers involved in oxidative stress and also may act as inhibitor of free radicals mediated oxidative damages as primary antioxidant. The HA of *T. chebula* extract displayed efficient activities due to presence of high quantitities of phenolic compounds as identified by chromatographic techniques. Further the bioactive constituents present in the extract should be isolated and characterized for determination of the *in vivo* protective effects.

## ACKNOWLEDGMENT

Authors are thankful to SRM University, for providing all necessary facilities.

## REFERENCES

Bag, A., S.K. Bhattacharyya and R.R. Chattopadhyay, 2013. The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research. Asian Pac. J. Trop. Biomed., 3: 244-252.

Chanput, W., J.J. Mes and H.J. Wichers, 2014. THP-1 cell line: An *in vitro* cell model for immune modulation approach. Int. Immunopharmacol., 23: 37-45.

Chen, X., F. Sun, L. Ma, J. Wang, H. Qin and G. Du, 2011. *In vitro* evaluation on the antioxidant capacity of triethylchebulate, an aglycone from *Terminalia chebula* retz fruit. Indian J. Pharmacol., 43: 320-323.

Cheng, H.Y., T.C. Lin, K.H. Yu, C.M. Yang and C.C. Lin, 2003. Antioxidant and free radical scavenging activities of *Terminalia chebula*. Biol. Pharm. Bull., 26: 1331-1335.

Garcia-Nino, W.R. and C. Zazueta, 2015. Ellagic acid: Pharmacological activities and molecular mechanisms involved in liver protection. Pharmacol. Res., 97: 84-103.

Gusman, G.S., P.R. Campana, L.C. Castro, R.O. Castilho, M.M. Teixeira and F.C. Braga, 2015. Evaluation of the effects of some brazilian medicinal plants on the production of TNF-α and CCL2 by THP-1 cells. Evid Based Complement. Alternat. Med. 10.1155/2015/497123

Kam, A., K.M. Li, V. Razmovski-Naumovski, S. Nammi, K. Chan and G.Q. Li, 2014. Gallic acid protects against endothelial injury by restoring the depletion of DNA methyltransferase 1 and inhibiting proteasome activities. Int. J. Cardiol., 171: 231-242.

Kathirvel, A. and V. Sujatha, 2012. *In vitro* assessment of antioxidant and antibacterial properties of *Terminalia chebula* retz. leaves. Asian Pac. J. Trop. Biomed., 2: S788-S795.

Krejsa, C.M. and G.L. Schieven, 2000. Detection of oxidative stress in lymphocytes using dichlorodihydrofluorescein diacetate. Methods Mol. Biol., 99: 35-47.

Lee, H.H., K.R. Paudel and D.W. Kim, 2015. *Terminalia chebula* fructus inhibits migration and proliferation of vascular smooth muscle cells and production of inflammatory mediators in RAW 264.7. Evid Based Complement. Alternat. Med. 10.1155/2015/502182

Lee, W.J., H.C. Ou, W.C. Hsu, M.M. Chou and J.J. Tseng *et al*., 2010. Ellagic acid inhibits oxidized LDL-mediated LOX-1 expression, ROS generation and inflammation in human endothelial cells. J. Vascular Surg., 52: 1290-1300.

Lin, L.T., W.C. Hsu and C.C. Lin, 2014. Antiviral natural products and herbal medicines. J. Tradit Complement. Med., 4: 24-35.

Lv, L., L. Zheng, D. Dong, L. Xu and L. Yin *et al*., 2013. Dioscin, a natural steroid saponin, induces apoptosis and DNA damage through reactive oxygen species: A potential new drug for treatment of glioblastoma multiforme. Food Chem. Toxicol., 59: 657-669.

Maddinedi, S.B., B.K. Mandal, R. Vankayala, P. Kalluru and S.R. Pamanji, 2015. Bioinspired reduced graphene oxide nanosheets using *Terminalia chebula* seeds extract. Spectroc. Acta Part A: Mol. Biomol. Spectrosc., 145: 117-124.

Mathew, M. and S. Subramanian, 2014. *In vitro* screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders. PLoS One, Vol. 9. 10.1371/journal.pone.0086804

Rekha, V., Jayamathi, RamaKrishnan, D. Vijayalakshmi and Prabu *et al*., 2014. Anti cariogenic effect of *Terminalia chebula*. J. Clin. Diagn. Res., 8: ZC51-ZC54.

Saha, S. and R.J. Verma, 2014. Antioxidant activity of polyphenolic extract of *Terminalia chebula* retzius fruits. J. Taibah Univ. Sci. 10.1016/j.jtusci.2014.09.003.

Sham, T.T., C.O. Chan, Y.H. Wang, J.M. Yang, D.K. Mok and S.W. Chan, 2014. A review on the traditional Chinese medicinal herbs and formulae with hypolipidemic effect. Biomed. Res. Int. 10.1155/2014/925302.

Singhal, M., A. Paul and H.P. Singh, 2014. Synthesis and reducing power assay of methyl semicarbazone derivatives. J. Saudi Chem. Soc., 18: 121-127.

Subramanian, V., B. Venkatesan, A. Tumala and E. Vellaichamy, 2014. Topical application of Gallic acid suppresses the 7,12-DMBA/Croton oil induced two-step skin carcinogenesis by modulating anti-oxidants and MMP-2/MMP-9 in Swiss albino mice. Food Chem. Toxicol., 66: 44-55.

Vasanth, K., K. Ilango, R. MohanKumar, A. Agrawal and G.P. Dubey, 2014. Anticancer activity of *Moringa oleifera* mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction. Colloids Surf. B: Biointerfaces, 117: 354-359.

Wang, J., J.R. Du, Y. Wang, X. Kuang and C.Y. Wang, 2010. Z-ligustilide attenuates lipopolysaccharide-induced proinflammatory response via inhibiting NF-κB pathway in primary rat microglia. Acta Pharmacol. Sin., 31: 791-797.