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Evaluation of *in vitro* antioxidant and cytotoxic activity study of 2-thiohydantoin

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***Abstract*:The thiohydantoin nucleus is a 5-membered ring system containing a reactive cyclic thiourea core. This heterocycle is used for the synthesis of drugs with antidiabetic, antimicrobial, anticancer, anticonvulsant, antiinflammatory, antiulcer and antiarrhythmic agents. The aim of the study was to evaluate the antioxidant and cytotoxic activity of the synthetic compound, 2-thiohydantoin. The present study gives information regarding four different *in vitro* methods that were used to measure the antioxidant activity of the synthetic compound, 2-thiohydantoin. *In vitro* studies on the effect of 2-thiohydantoin on scavenging 2,2-diphenyl-1-picrylhydrazl radical(DPPH$^{\bullet}$), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation (ABTS$^{\bullet}$), hydroxyl radical (OH$^{\bullet}$) and superoxide anion radical ($O_2^{\bullet-}$) confirmed the free radical scavenging and antioxidant activity of 2-thiohydantoin. The cytotoxic activity of 2-thiohydantoin on L6 & 3T3-L cells was determined using MTT assay. The IC50 values of 2-thiohydantoin on L6 and 3T3-L cells was 21.25 µg and 25.25 µg respectively. These results indicate that the synthetic compound, 2-thiohydantoin has good antioxidant and significant cytotoxic activity.**

***Keywords*: thiohydantoin, antioxidant, cytotoxic, free radical, DPPH.**

1. INTRODUCTION

A free radical is a molecule that is capable of independent existence and contains an unpaired electron in an atomic orbital. Free radicals are unstable and highly reactive species. Free radicals are produced in our body as a part of normal metabolic process. They possess both harmful and beneficial effects [1]. Overproduction of reactive oxygen species (ROS) are highly reactive and toxic causing damage to macromolecules such as proteins, lipids, DNA and carbohydrates leading to oxidative stress. This oxidative stress causes tissue damage and results in many diseases such as neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases [2],[3]. Antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being [4].

Antioxidants can be natural or synthetic. Natural antioxidants are present in the diet especially in fruits, vegetables and spices. Antioxidants include enzymes such as superoxide dismutase (SOD), catalase(CAT) and glutathione reductase(GR) and minerals like Se, Cu, Zn etc. Also vitamin C, vitamin E, carotenoids and flavonoids neutralize the effects of ROS and thus help in preventing diseases [5]. Recently scientists have found that synthesized molecules such as derivatives of hydantoins possess important biochemical and pharmacological properties. Compounds possessing hydantoin moiety provides a platform for the discovery of new drugs because of their cyclic analogy to natural aminoacids. The derivatives of hydantoins have shown antioxidant activity and are able to protect the body from oxidation [6], [7]. The cytotoxicity of the hydantoins are also investigated for medical and clinical applications.[8].

The purpose of the present study is to evaluate the antioxidant activity of 2-thiohydantoin and to examine whether the synthetic compound 2-thiohydantoin shows significant cytotoxic activity.

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2. MATERIALS AND METHODS

Sample

The synthetic compound, 2-thiohydantoin ($C_3H_4N_2OS$) is available as brown crystalline powder having molecular weight of 116.14. The drug was purchased from SIGMA-ALDRICH, Germany. The parent compound hydantoin was first isolated in 1861 by Adolf von Baeyer in the course of his study of uric acid. He obtained it by hydrogenation of allantoin, hence the name. 2-thiohydantoin is also known by other names like 4-Imidazolidinone, 2-thioxo-; 2-thioxoimidazolidin-4-one and 2-Thioguidanthion. The compound was soluble in dimethyl sulfoxide (DMSO).

DPPH scavenging assay

The antioxidant activity of 2-thiohydantoin was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams et al.[9] with slight modifications. The samples containing the compound 2-thiohydantoin was reacted with the ethanolic extract of DPPH. Twenty minutes later the absorbance was measured at 517 nm using UV visible spectrophotometer. IC50 was determined. The activity is expressed as the inhibitory concentration IC50, the value denoting the concentration of sample required to scavenge 50% of the DPPH free radicals. IC50 value was calculated from % inhibition which was calculated from the following formula.

% inhibition = (A of blank - A of Test)/A of blank X 100,

where A indicates the absorbance value.

ABTS scavenging assay

This assay measures the ability of 2-thiohydantoin to scavenge 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid(ABTS •+) radical cation as described by Emad A. Shalaby and SanaaM. M. Shanab with slight modificaitons[10]. The radical cation was prepared by reacting 0.002M ABTS with 0.07M potassium persulphate and the mixture was allowed to stand in dark room temperature for 12-16 hours before use. The absorbance was read at 734nm using UV VISIBLE spectrophotometer and the % inhibition was calculated.

% inhibition = (A of blank - A of Test)/A of blank X 100

where A indicates the absorbance value.

IC50 values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC50 was calculated for all the extracts based on the percentage of ABTS radicals scavenged. Reduction of blue-green ABTS radical coloured solution by hydrogen donating sample compound was measured.

Hydroxyl radical scavenging assay

The scavenging activity for hydroxyl radicals was measured using Fenton reaction as described by Man Kyu Huh et.al with slight modifications [11]. Sample to be tested was taken at different concentrations (10-50µg/ml).The reaction mixture contains 0.2ml of ferric chloride, 0.1ml of Ascorbic acid, 0.1ml of EDTA, 0.2 ml of 2-deoxyribose, 1.0 ml of thiobarbutiric acid 1.0 ml of Trichloroacetic acid and 0.1ml of Hydrogen peroxide. After incubation at room temperature for 5 minutes, the absorbance of the mixture (yellow colour) at 532 nm was measured using UV-Visible spectrophotometer. The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

% Inhibition = (A of blank - A of Test)/A of blank X 100

where A indicates the absorbance value. IC50 values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC50 was calculated for all the extracts based on the percentage of hydroxyl radical (OH•) scavenged.

Superoxide anion scavenging assay

The superoxide radical scavenging activity of the sample was investigated using phenazinmethosulphate(PMS)-Nicotinamide adenine dinucleotide (NADH)-nitrobluetetrazolium(NBT) system as described by F.Liu et al. with slight modifications[12]. The reaction mixture was incubated for 30 min and the absorbance at 560 nm was measured againstthe

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control samples using UV-VISIBLE spectrophotometer. The percentage inhibition was calculated by comparing the results of control and test samples.

% Inhibition = (A of blank - A of Test)/A of blank X 100

where A indicates the absorbance value.

IC50 values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC50 was calculated for all the extracts based on the percentage of superoxide anions scavenged.

MTT assay

The cytotoxic activity of the sample on L6 (rat myoblast cell line) and 3T3-L (rat fibroblast cell line) cells was determined by the (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) MTT assay as described by T.Mosmannet.al., 1983[13]. Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates. The cells were incubated at 5 % CO_2 incubator for 72 hours. Then, added various concentrations of the samples in 0.1% DMSO for 24hrs at 5 % CO_2 incubator. Then the images were viewed under inverted microscope 40X and the photos were taken. After removal of the sample solution and 20µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of dimethyl sulfoxide(DMSO) was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the samples on the proliferation of L6 & 3T3-L cells was expressed as the % cell viability, using the following formula:

Calculation

% cell viability = A540 of treated cells / A540 of control cells × 100%

3. RESULTS AND DISCUSSION

The DPPH assay measured hydrogen atom (or one electron) donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. DPPH is a purple coloured free radical and it was reduced into the yellow-coloured diphenylpicryl hydrazine which was measured spectrophotometrically at 517nm.

The dose response curve of DPPH radical scavenging activity of 2-thiohydantoin was observed and shown in Fig.1. The free radical scavenging was found to be increased as the concentration of the drug increases. Lower absorbance of the reaction mixture showed higher free radical scavenging activity. The Inhibition concentration, IC50 was calculated by plotting percentage inhibition against different concentrations of the compound as depicted in Fig.5. The Inhibition concentration IC50 value of DPPH scavenging assay was found to be 30. In DPPH assay, the drug of interest, 2-thiohydantoin was able to reduce the violet coloured stable 1, 1- diphenyl-2-picryl hydrazyl radical to the yellow coloured 1, 1- diphenyl-2-picryl hydrazine. Sulfur containing compounds such as 2-thiohydantoin readily undergo the redox reactions by the opening of the dithiol ring and the free radicals react with thione double bond. 2-thiohydantoin possess only one sulphur group and shows the electron donating property to scavenge the free radicals formed during the reaction.[20]

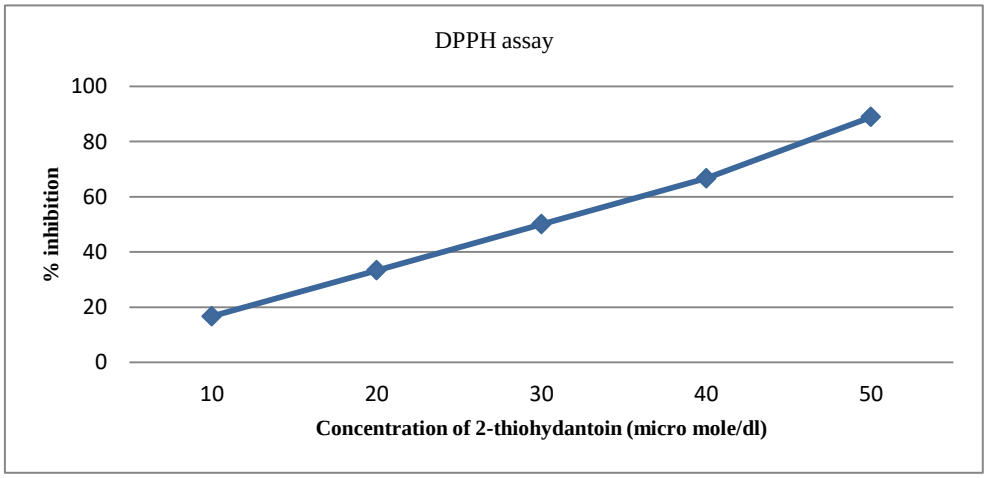

Fig.1 DPPH assay

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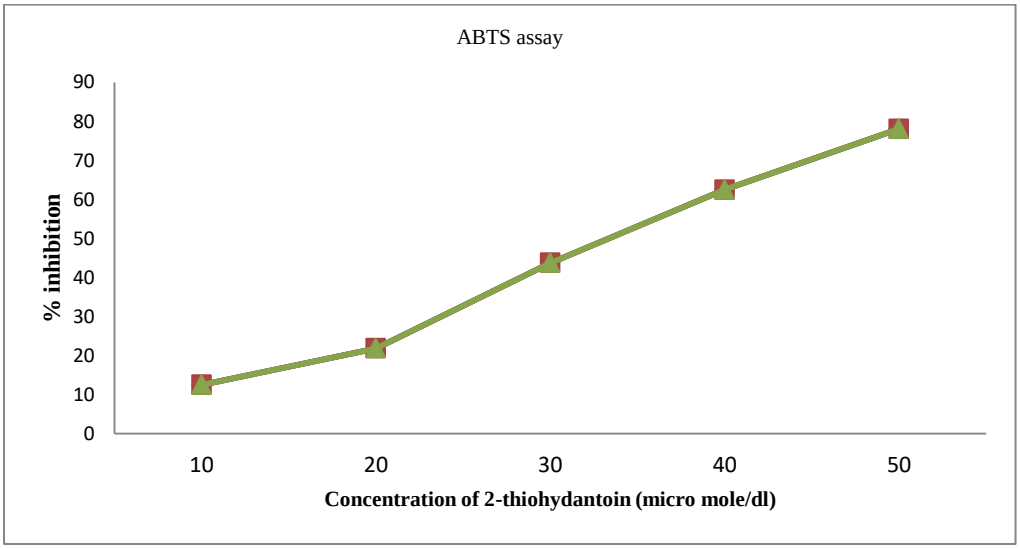

Fig.2. ABTS assay

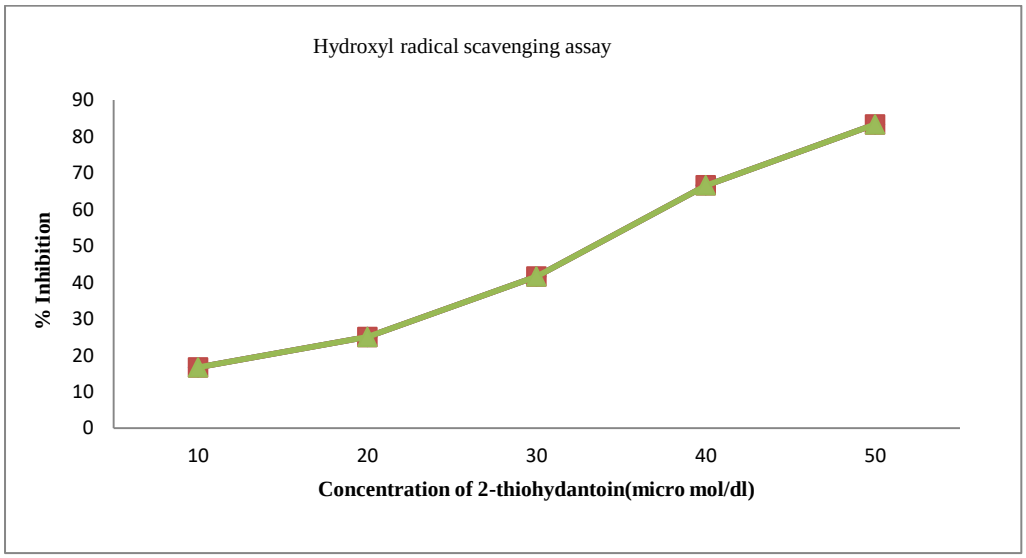

Fig.3. Hydroxyl radical scavenging assay

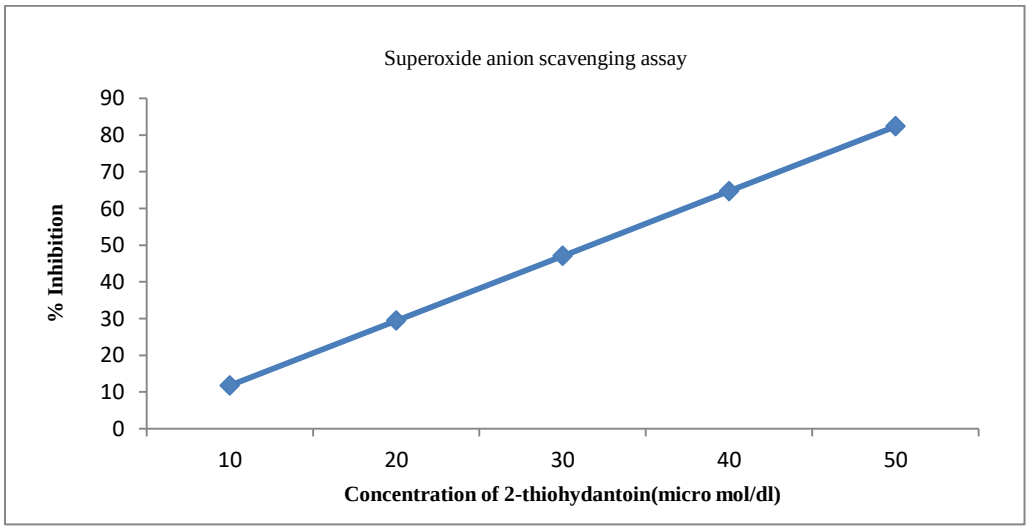

Fig.4. Superoxide anion scavenging assay

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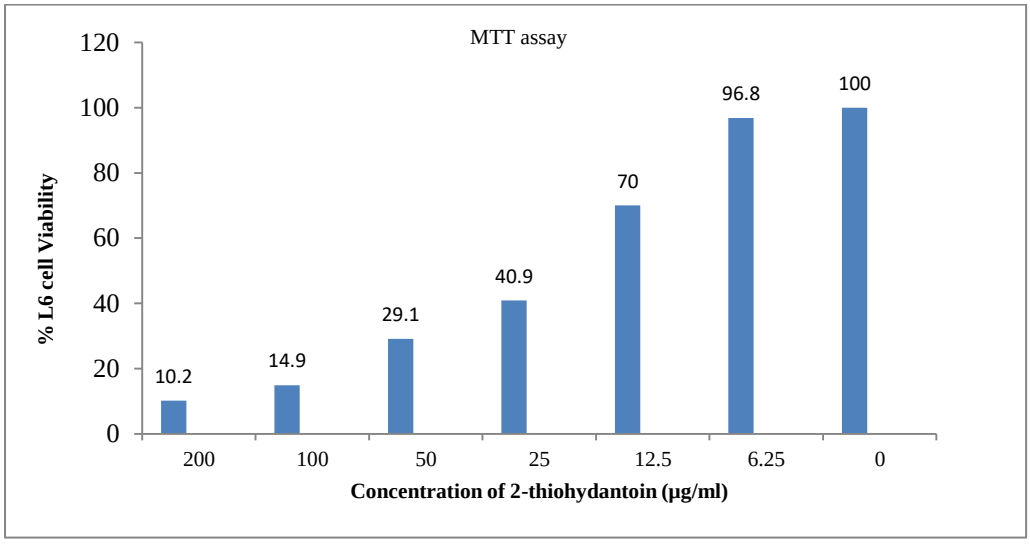

Fig.5.a. MTT assay [L6 Cell viability]

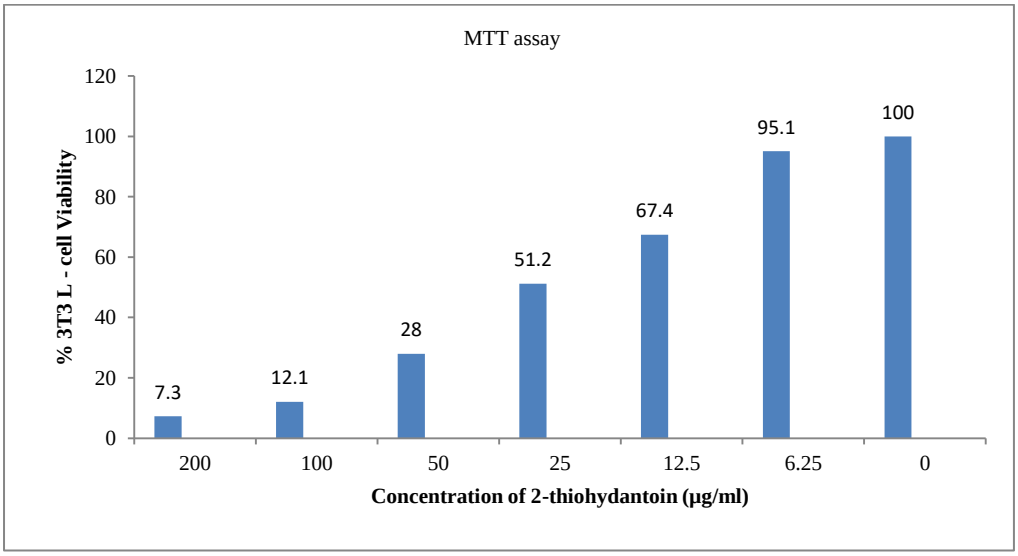

Fig.5.b. MTT assay [3T3L-Cell viability]

The ABTS cation radical (ABTS •+) was formed by the loss of an electron by the nitrogen atom of ABTS. ABTS was oxidised by potassium per sulphate, giving rise to ABTS cation radical (ABTS •+). Then it was reduced in the presence of the sample compound, 2-thiohydantoin, a hydrogen-donating antioxidant. [14]. Antioxidant activity was calculated by plotting percentage inhibition against different concentrations of compound. The dose response curve of ABTS •+ radical scavenging activity of 2-thiohydantoin was observed and shown in Fig.2. The antioxidant activity of the drug was found to be increased as the concentration of the sample increases. The Inhibition concentration IC50 value of ABTS cation radical scavenging assay was found to be 36.34 [Fig.2].

Fenton reaction is a convenient generator of the hydroxyl radical with known implications in health and disease .The reaction traditionally involves reduction of H2O2 with Fe (II), but can also occur in presence of copper. [15] The dose response curve of ABTS cation radical scavenging assay of 2-thiohydantoin was observed and shown in Fig. 3. It show that the higher concentration of the sample exhibited strong scavenging abilities for the hydroxyl radical. The Inhibition concentration IC50 value of hydroxyl radical scavenging assay was calculated by plotting percentage inhibition against the concentrations of the sample as depicted in Fig.3. IC50 value of hydroxyl radical scavenging was found to be 38.78.

Superoxide is biologically quite toxic and is deployed by the immune system to kill invading microorganisms. It is an oxygen-centred radical with selective reactivity. Superoxide anion plays an important role in the formation of other ROS(reactive oxygen species) such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative

damage in lipids, proteins and DNA. Superoxide radical is normally formed first, and its effects can be magnified because it produces other kinds of free radicals and oxidizing agents [16]. In this method, superoxide anion reduces the yellow dye (NBT2+) to produce the blue formazan which is measured spectrophotometrically at 560 nm. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Figure 4 shows the scavenging activity percentage of superoxide radical by the sample, 2-. Thiohydantoin. The Inhibition concentration IC50 value of hydroxyl radical scavenging assay was calculated graphically by plotting percentage inhibition against different concentration of the sample as shown in the Fig.4. IC50 value of superoxide anion scavenging was found to be 32. Ascorbic acid was used as a standard reference drug (positive control) for all the antioxidant assays.

The well-described colorimetric tetrazolium salt (MTT) assay, which monitors metabolic activity of cultured cells, was adapted to analyze the viability of cells[18] .The assay relies on the reduction of MTT, a yellow water-soluble tetrazolium dye, primarily by the mitochondrial dehydrogenases, to purple colored formazan crystals. The formazan product is analyzed spectrophotometrically (550 nm) after dissolution in DMSO, giving an estimate of the extent of cytotoxicity [19].The sample compound was found to inhibit the cell viability as observed in the Fig 5.a and Fig. 5.b. The IC50 was calculated graphically for the L6 cells were found to be 21.25µg and 3T3-L cells was found to be 25.25µg. As the concentration increases the drug was found to inhibit the cell viability and shows cytotoxic activity.

4. CONCLUSION

In this study the antioxidant activity and cytotoxic effect of 2-thiohydantoin were examined. The results shows that the sample compound, 2-thiohydantoin possess good antioxidant activity and significant cytotoxic effect. These results suggest that the drug 2-thiohydantoin can be used for the development of new medicinal drugs to cure diseases.

REFERENCES

[1] Ganesh T, SaikatSen , Raja Chakraborty, Suresh Kumar SV et al, "*In vitro* antioxidant activity of Meynalaxiflora seeds", International Journal of Chemical and Pharmaceutical Sciences, Aug., vol.1 (1), 2010.

[2] VinitaSindhia, VartikaGuptaa, Kameshwar Sharma et al, "Potential applications of antioxidants – A review", Journal of Pharmacy Research, vol.7, Issue 9, pp.828-835, September 2013.

[3] HarsahayMeena, Hemant Kumar Pandey, PankajPandey et al, "Evaluation of antioxidant activity of two important memory enhancing medicinal plants Baccopamonnieri and Centellaasiatica", Indian J Pharmacol. 2012 Jan-Feb; 44(1): pp.114–117.

[4] BanditaDeo, MonalishaNath, Preetam Kumar Nayak, Yogamaya Dhal, "Evaluation of antioxidant activity of Ocimumtenuiflorum, an important medicinal herb", International journal of plant, animal and environmental sciences, vol.3, Issue 2, April-June2013.

[5] Vinay R. Patel, Prakash R. Patel and Sushil S. Kajal, "Antioxidant Activity of Some Selected Medicinal Plants in Western Region of India", Advances in Biological Research 4 (1): pp.23-26, 2010.

[6] Yan A.Ivanenkov, Mark S.Veselov et al, "Synthesis, isomerization and biological activity of novel 2-selenohydantoin derivatives", Bioorganic & Medicinal Chemistry, vol. 24, Issue 4, 15 February 2016, pp. 802-811.

[7] KatarzynaSladowska, Jadwiga Handslik et al, "*In vitro* cytotoxic activity evaluation of phenytoin derivatives against human leukemia cells, Indian Journal of Experimental Biology, vol.54, Sep 2016, pp.553-559.

[8] PetjaMarinova, Marin Marinov et al, "Synthesis, cytotoxicity and antibacterial activity of 3-amino-9'-fluorenespiro-5-hydantoin", Compt. rend. Acad. bulg. Sci., 67, No 4, 2014.

[9] W. Brand-Williams, M. E. Cuvelier , C. Berset , "Use of a Free Radical Method to Evaluate Antioxidant Activity", Lebensm.-Wiss. u.-Technol., 28. pp.25-30,1995.

[10] Emad A. Shalaby, Sanaa M.M. Shanab, "Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulinaplantensis", Indian Journal of Geo-Marine Sciences, vol.42(5), Sep.2013, pp.556-564.

[11] Man Kyu Huh, Chin Bum Lee and Sung Gi Moon, "Inhibitory Effect of DPPH Radical Scavenging Activity and Hydroxyl Radicals (OH) Activity of Chelidoniummajus var. asiaticum", International Journal of Advanced Multidisciplinary Research,vol.3, Issue.3, pp.15-19, 2016.

[12] Liu F1, Ooi VE, Chang ST, "Free radical scavenging activities of mushroom polysaccharide extracts", Life Sci. 1997;60(10):pp.763-71.

[13] Mosmann T, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays", J Immunol Methods. 1983 Dec 16; 65(1-2):pp.55-63.

[14] RobertaRea, NicolettaPellegrinia, AnnaProteggente et al, "Antioxidant activity applying an improved ABTS radical cationdecolorization assay", Free Radical Biology and Medicine, vol. 26, Issues 9–10, May 1999, pp.1231-1237.

[15] S. A. Mir, A. S. Bhat , A. A. Ahangar, "A new Fenton assay for Hydroxyl radical scavengers by monitoring Catechol oxidation", International Journal of PharmTech Research, vol.6, No.2, pp 759-768, April-June 2014.

[16] IlhamiGulcin, ZubeyrHuyut et al, "Radical scavenging and antioxidant activity of tannic acid", Arabian Journal of Chemistry, vol. 3, Issue 1, January 2010, pp. 43-53.

[17] Sucheta A Gaikwad, Gayatri S. Kamble, Swati Devare et al, "Journal of Chemical and Pharmaceutical Research", 2011, 3(4): pp.766-772.

[18] Hansen J, Bross P, "A cellular viability assay to monitor drug toxicity", Methods Mol Biol. 2010;648:pp.303-11.

[19] VandanaPatravale, PrajaktaDandekar, Ratnesh Jain, "4 – Nanotoxicology: evaluating toxicity potential of drug-nanoparticles", Nanoparticulate Drug Delivery, 2012, pp. 123–155.

[20] Kiec-Kononowicz K, Karolak-Wojciechowska J, Robak J, "Fused 2-thiohydantoin derivatives: evaluation as potential antioxidants", Arch Pharm (Weinheim). 1997 Apr;330(4):pp.85-90.