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ORIGINAL ARTICLE **OPEN ACCESS**

In vitro Antioxidant activity of Leaf extract of *Melia azedarach* L. in avian lymphocytes

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ABSTRACT
There is a renewed interest in herbal medicines. Medicinal plants are rich in various phytochemicals that includes polyphenols and carotenoids responsible for beneficial effects on health. The polyphenols and carotenoids are having antioxidative potential which makes medicinal plants a source of natural antioxidants. Free radicals produced during metabolic process produces various pathological conditions. The antioxidants are able to prevent the damage caused by these free radicals. Melia azedarach is potent medicinal plants reported to have various medicinal and therapeutic effects. Leaves of Melia azedarach is traditionally used to cure various diseases viz. malaria, diabetes, cough, skin diseases etc. In the present study, the antioxidative potential of hydromethanolic leaves extract of Melia azedarach (MAE) was explored through DPPH and NO radical scavenging assays. The antioxidative potential of the extract was estimated after in vitro exposure of MAE to chicken lymphocytes through various assays viz. membrane lipid peroxidation (LPO), reduced glutathione (reduced GSH), Superoxide dismutase (SOD) and catalase. Content of cellular nitric oxide was also estimated in chicken macrophages rich population after MAE treatment. MAE showed significant antioxidative activity in both DPPH and Nitric Oxide (NO) radical scavenging activity. Nitric oxide content and membrane lipid peroxidation was decreased in MAE treated cells in comparison to control untreated cells. However, SOD, reduced GSH and catalase was increased significantly after MAE treatments as compared to control. In conclusion, this study showed that hydromethanolic leaves extract of Melia azedarach (MAE) showed marked antioxidative potential and it could be a potential source of natural plant antioxidants.
Keywords: *Melia azedarach; chicken lymphocyetes; antioxidant; nitric oxide; DPPH assay.*

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INTRODUCTION

In living organism, due to various on-going metabolic processes various reactive oxygen species (ROS) are generated. The ROS are basically, free radicals of oxygen having one or more unpaired electron which reacts with other molecules. At higher concentration these ROS can cause damage to essential molecules of body like DNA, lipids and proteins etc. which leads to generation of "Oxidative stress" [29]. Due to oxidative stress various pathological conditions like cancer, neurological disorders, atherosclerosis, hypertension, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, asthma could be generated [6]. Antioxidants are any substances or compounds that are able to counteract the effects of free radicals (ROS) at low concentration and prevent the occurrence of cancer and various pathological conditions related to ROS [10]. Natural occurring antioxidants are vitamin C and E, lipoic acid, beta carotene which are present in various green leafy vegetables and synthetic antioxidants are Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and Ethoxyquin (ETO), etc. and are used as food additives for preservation. Synthetic antioxidants have carcinogenetic effects, which makes them undesirable for consumption as antioxidants [20].

Natural antioxidants are preferred over synthetic antioxidants for being safe in nature. There has been increasing concerns of medicinal plants due to their extensive therapeutic and antioxidative properties. Medicinal plants have various phytochemicals and constituents which are secondary metabolites responsible for their antioxidative potential [14, 5]. *Melia azedarach L.* (Bakain) belong to family Meliaceae which is native to tropical Asia. Traditionally, different parts of the plants *i.e.* leaf, flower, seed,

fruit, young branches has been used for treatment of malaria, diabetes, cough, skin diseases etc. *Melia azedarach* also reported to have the antioxidant, antimicrobial, anti-inflammatory, cardio protective, analgesic, anticancer, antiulcerative properties which are proven by experimental and clinical studies [2]. Thus the present study was conducted to explore the antioxidative potential of *Melia azedarach* leaves through *in vitro* biochemical assays as well as different cell based antioxidant assays after *in vitro* exposure of MAE to chicken lymphocytes.

MATERIAL AND METHODS

Plant material

The authentic plant material *i.e.*, leaves of *Melia azedarach* were obtained from Medicinal Plant Research and Development Centre (MRDC), Pantnagar, Uttrakhand, India.

Extract preparation

Extract was prepared by following the method of Thakur *et al.* [28] with few modifications. The leaves of *Melia azedarach* were washed thoroughly with running tap water and finally with distilled water. It was then shade dried and on complete drying it was ground to make a fine powder in a grinder. 100 gram of this shade dried powder was added to 500 ml of methanol and autoclaved distilled water (50% v/v) and kept for 48 hours under continuous agitation in a shaking incubator. Afterwards it was filtered through muslin cloth and then through Whatmann filter paper no 1. The aqueous extract was rotary evaporated at 45°C to evaporate the solvent and then subjected to freeze drying. Finally the extract was obtained after lyophilisation, weighed and stored in airtight containers at -20^{0}C deep freezer till further use.

Evaluation of antioxidative potential of MAE

DPPH assay

The DPPH assay was carried out by following the method of Shivhare *et al*. [24]. For DPPH assay, 100µl of various concentrations of the plant extract dissolved in distilled water ranging from 5-100µg/ml and 100µl of 0.3mM DPPH solution in methanol was added to 96 well plates in triplicates. Plates were kept at 30^{0}C for 30 min and the absorbance was measured at 517 nm. Ascorbic acid was used as a positive control. Percent DPPH radical scavenging activity was calculated according to decrease in absorbance at 517nm. The formula used for calculation is as follows:

$$Percentage\ (\%) DPPH\ scavenging\ = \frac{(Abs\ of\ control - Abs\ of\ sample)}{Abs\ of\ control} \times 100$$

The IC_{50} values were calculated from regression curve and statistical analysis was done.

Nitric Oxide radical scavenging assay

Nitric Oxide (NO) radical scavenging assay was carried out by following the method Jagetia *et al* [11]. For NO radical scavenging assay, sodium nitroprusside was used to generate the free radicals which was measured by Griess reagent.10mM sodium nitroprusside in phosphate buffer saline was prepared. Plant extract at various concentrations ranging from 10-100µg/ml was used. 100µl of each concentration of plant extract was added to 96 well plates in triplicate. The plate was incubated for 150 minutes at 25^{0}C in dark. 100µl of Griess solution was added in each well of 96 well plate and absorbance was measured at 546 nm. Ascorbic acid was used as a positive control. Percent NO radical scavenging activity was calculated according to the absorbance at 546 nm. The IC50 values were calculated from regression curve and statistical analysis was done. The formula used for calculation is as follows:

$$Percentage\ (\%) NO\ scavenging = \frac{(Abs\ of\ control - Abs\ of\ sample)}{Abs\ of\ control} \times 100$$

Isolation of chicken lymphocytes

Chicken spleens were collected from healthy chickens (White Leghorn) of the age of 5-7 weeks in sterile Dulbecco's phosphate buffer saline from local slaughterhouse and brought to the laboratory. Then spleens were further processed immediately to isolate lymphocytes under strict aseptic conditions. Lymphocytes from chicken spleens were isolated under laminar air flow as per standard procedure of by Janossy and Greaves [12]. Lymphocytes were separated through density gradient centrifugation (Hisep, Himedia) as per the Method described by Rose and Friedman [22].

Nitric oxide estimation from macrophage culture

Microplate assay method described by Stuehr and Nathan [26] was performed for nitric oxide estimation produced by macrophages in medium. After density gradient centrifugation, cells isolated from chicken spleen was plated in 6 well plates and incubated for 2 hour at 37^{0}C in a CO_2 incubator. Then plate was washed with media twice for removal of non-adherent cells. Fresh medium and extract was added in plate. Plate was incubated for 68 hours at 37^{0}C in CO_2 incubator.100µl of supernatant fluid was removed

from well in an ELISA plate and incubated with equal volume of Griess reagent (Sigma, USA) at room temperature for 30 min in dark. The absorbance at 548 nm wavelength was determined in Biotek ELISA plate reader. NO was determined by using sodium nitrite ($NaNO_2$) as a standard.

Biochemical Assays

For determination of antioxidant potential of *Melia azedarach* in chicken lymphocytes various enzymatic antioxidative assays were performed. The maximum non cytotoxic dose of MAE in chicken lymphocytes was determined by exposing cells with various dilutions of MAE and the dose was determined as 150µg/ml which was further used to give *in vitro* exposure to chicken lymphocytes and to determine the antioxidative status of the exposed cells [7]. The activity of enzymes in the antioxidant system was evaluated in the MAE exposed cells. After exposure cells were harvested and cell lysate was prepared and subjected to different antioxidant assays [25].

Membrane lipid peroxidation

The level of membrane lipid peroxidation (LPO) was evaluated by following the TBARS assay of Ohkawa *et al.* [19]. The end product of membrane peroxidation is Malondialdehyde (MDA) which was measured. MDA reacts with thiobarbituric acid and generate a coloured product, which is measured by taking absorbance at 532 nm wavelength.

Reduced glutathione

The level of reduced glutathione was estimated as per the method described by Ellman [9]. Reduced glutathione (GSH) is measured by its reaction with DTNB (5,5′-dithiobis-2-nitrobenzoic acid) (Ellman's reaction) to give a yellow coloured product that absorbs at 412 nm. .

Superoxide dismutase

The superoxide dismutase was estimated by following the method of Madesh and Balasubramanian [15]. It involved generation of superoxide by pyrogallol auto oxidation and the inhibition of superoxide dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethylthiazol 2-xl) 2, 5-diphenyl tetrazolium bromide] to its formazan, which was measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helped to solubilize formazan formed during the reaction.

Catalase

Catalase was estimated as per the method of Aebi [1]. Catalase catalysed the decomposition of H_2O_2. In the ultraviolet range H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in extinction at 240 nm.

RESULTS AND DISCUSSION

Extraction Yield

Hydromethanolic extract of leaves of *Melia azedarach* (MAE) was prepared and the percent yield of MAE was found out to be 12.72%.

DPPH assay

Through DPPH assay antioxidative potential of the extract was determined by scavenging the radicals generated by DPPH. The extract showed antioxidative potential by scavenging DPPH radicals however it was found to be much less than the scavenging potential of ascorbic acid (Table 1 and Figure 1). Ascorbic acid is very well explored and well known antioxidant which was used as positive control in this assay. Ascorbic acid showed IC_{50} value of 7.02µg/ml while MAE showed IC_{50} as 119.56 µg/ml. lower IC_{50} value correspond to better antioxidant potential.

Nitric oxide radical scavenging assay

In this assay, antioxidative potential of the extract was determined by scavenging the NO radicals generated by sodium nitroprusside in aqueous solution. The extract showed antioxidative potential by scavenging NO radicals. Ascorbic acid showed IC_{50} value of 113.03µg/ml while MAE showed IC_{50} 114.31 µg/ml. Different concentrations of extract and ascorbic acid used to explore antioxidative activity. Data is presented in Table 2 and Figure 2. Dose dependent enhancement in the scavenging activity of MAE was observed.

Nitric oxide estimation

Nitric oxide content is calculated from the sodium nitrite standard curve. Nitric oxide content was determined after the exposure of MAE in macrophage rich cells (Table 3 and Figure 3). Ascorbic acid was taken as positive control and untreated cells were taken as normal control. MAE treated cells displayed 32.62µM NO content which was found to be significantly less than the NO content of control untreated as well as ascorbic acid treated cells which is indicative of good antioxidant potential of MAE.

Membrane lipid peroxidation

Lymphocytes were exposed to maximum non cytotoxic dose of MAE which was 150µg/ml. Thiobarbituric acid-reactive substances (TBARS) assay was used to determine the degree of lipid peroxidation. More the antioxidative potential of the plant extract lower will be the activity of TBARS. The MAE show significant

decrease in lipid peroxidation as presented in Table 4 and Figure 4. Ascorbic acid was used as positive control and cells without extract treatment was taken as normal control.

Reduced glutathione

The content of reduced glutathione was estimated after MAE exposure to chicken lymphocytes. The level of reduced glutathione was significantly increased after MAE treatment as compared to control which is depicted in Table 5 and Figure 5. In this assay, ascorbic acid was used as positive control and untreated cells were used as control.

Superoxide dismutase

Chicken lymphocytes were exposed to MAE and superoxide dismutase content was estimated. SOD content in MAE treated lymphocytes is presented in Table 6 and Figure 6. Ascorbic acid was taken as positive control and untreated cells were taken as normal control. The level of SOD was increased in comparison to normal control.

Catalase

The catalase content was estimated after MAE exposure to lymphocytes. The content was increased significantly after MAE treatment as compared to control. Ascorbic acid treated cells showed maximum increase in catalase content (Table 7 and figure 7).Globally, the interest has been increased to identify the natural compounds with good antioxidative potential without any side effects for pharmacological usage. Medicinal plants or plants prevent the antioxidative stress by producing antioxidants by reacting with photons and oxygen [4]. In plants, antioxidants are basically phytochemicals which are present in form of phenolics, flavonoids, alcohols, stilbenes, tocopherols, tocotrienols, ascorbic acid and carotenoids [5, 30]. Various reports are available showing presence of different phytoconstituents in *Melia azedarach* that are responsible for medicinal properties of this plant [18, 2, 3, 23, 7, 8]. However, very few reports are available regarding antioxidative potential of this plant in *in vitro*/ *in vivo* animal model system. In this study, *in vitro* antioxidative potential of MAE was explored through DPPH and NO radical scavenging assay. MAE showed decrease in DPPH radical concentration with increase in the MAE concentration. Nitric oxide radical scavenging assay was also performed to detect antioxidative potential. Nitric oxide have unpaired electron which makes it NO radicals and these radicals reacts with oxygen to reactive nitrogen species involving NO_2, N_2O_4, N_3O_4 and nitrate, nitrite, peroxynitrite that are reacts with superoxide [16]. MAE showed significant dose dependent antioxidative activity, as the concentration of extract the scavenging activity was also increased.

Antioxidants are both enzymatic and non-enzymatic, in enzymatic antioxidant defense system includes membrane lipid peroxidation, reduced glutathione, superoxide dismutase and catalase. The cellular nitric oxide level was also estimated in this study. The cellular nitric oxide is produced endogenously by enzymes having three different isoforms iNOS (inducible NOS), eNOS (endotheial NOS), nNOS (neuronal NOS). Nitric oxide is a signalling molecule but at high concentration it reacts with oxygen and produces various reactive nitrogen species [17]. Macrophages rich population of chicken was exposed to MAE to estimate the nitric oxide content. The exposure of MAE caused decrease in the content of cellular nitric oxide as compared to control.

The enzymes react with various free radicals and prevent harmful effects of free radicals. Reactive oxygen species majorly reacts with membrane lipids and chain reaction imitated which result in membrane lipid peroxidation [13]. The level of membrane lipid peroxidation (LPO) was estimated through TBARS assay. After the exposure of MAE, the level of LPO was decreased which represent that extract having antioxidative potential by preventing the oxidation of lipids present in cellular membrane. Reduced glutathione content was measure, which is formed by Glutathione reductase using NADPH from pentose phosphate shunt pathway by converting the glutathione disulfide into reduced glutathione [2]. It was reported administration of ethanol, over a period of four weeks leads to oxidative stress, in rats, and the mixture of vitamins C and E significantly attenuated ethanol-induced changes. Methanolic leaf extract of *Melia azedarach* prevented the ethanol-induced changes in oxidative stress parameters, and effect was comparable to that of vitamins E and C [2]. The level of reduced GSH increased after the exposure of MAE as compare to control. Superoxide radicals are produced during mitochondrial respiration, the radicals reacts with oxygen to form ROS. Superoxide radicals react with metal ions present in catalytic sites of enzymes and make them inactive. Superoxide radicals are dismutated by superoxide dismutase into hydrogen peroxide and further these radicals are break down by catalase into water and oxygen [27].

The level of both superoxide dismutase (SOD) and catalase was estimated after MAE exposure in chicken lymphocytes. The level of both SOD and catalase was increased as compared to control. The increase in the level of these enzymatic antioxidants represents significant antioxidative potential of hydromethanolic leaves extract of *Melia azedarach*.

Table 1: Percent scavenging of MAE and ascorbic acid in DPPH assay

S. No.	Concentrations used (µg/ml)	Mean O.D. of MAE (517nm)	Mean O.D. of Ascorbic acid (517nm)	Percent Scavenging of MAE± SE	Percent Scavenging of Ascorbic acid ± SE
1	1	0.744	0.523	23.003±0.516	45.812±3.050
2	5	0.739	0.512	23.573±3.124	47.051±0.317
3	10	0.672	0.454	30.411±2.791	51.548±2.749
4	20	0.640	0.420	34.078±2.783	56.514±0.714
5	40	0.612	0.381	36.683±0.797	58.35±1.826
6	60	0.604	0.256	36.061±1.958	71.446±2.268
7	80	0.590	0.226	38.888±1.292	75.260±2.379
8	100	0.513	0.176	46.832±1.737	81.703±3.131
9	Control	0.967			
CD (5%)			SE(m)±		
6.285			2.181		

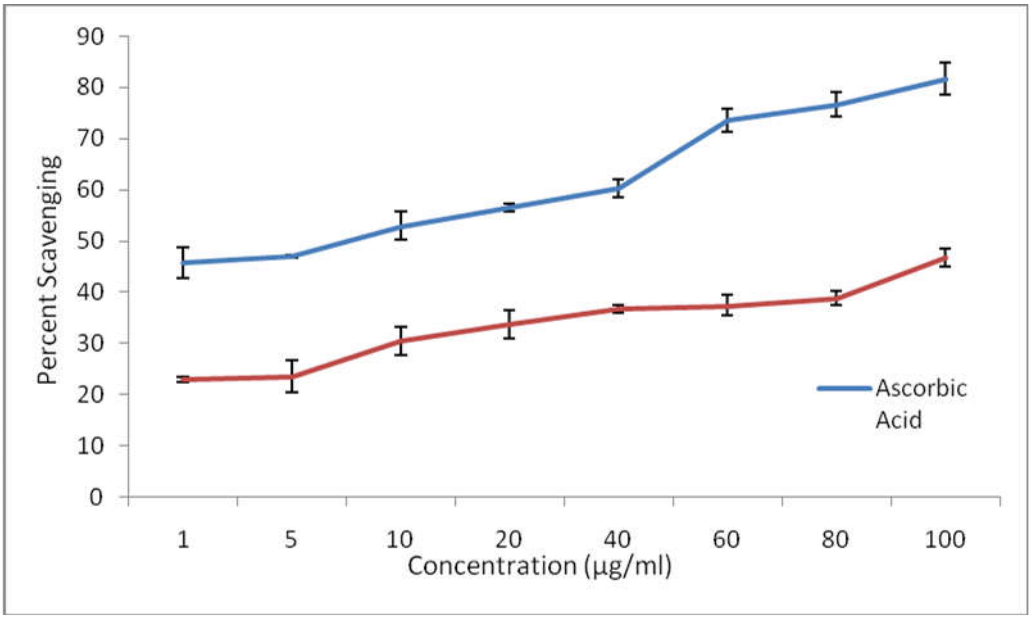

Figure 1: Percent Scavenging of MAE and Ascorbic acid in DPPH assay

Table 2: Percent scavenging of MAE and ascorbic acid in NO scavenging assay

S. No.	Concentrations used (µg/ml)	Mean O.D. of MAE (546nm)	Mean O.D. of Ascorbic acid (546nm)	Percent Scavenging of MAE± SE	Percent Scavenging of Ascorbic acid± SE
1	10	0.387	0.385	22.778±1.785	23.114±1.641
2	20	0.356	0.326	29.025±2.407	34.878±1.759
3	40	0.332	0.321	33.750±2.168	36.011±1.470
4	60	0.325	0.305	35.084±1.755	39.135±1.008
5	80	0.306	0.294	38.873±0.858	41.256±0.980
6	100	0.257	0.264	48.704±1.220	47.307±1.100
7	Control	0.501			
CD (5%)			SE(m)±		
1.584			4.625		

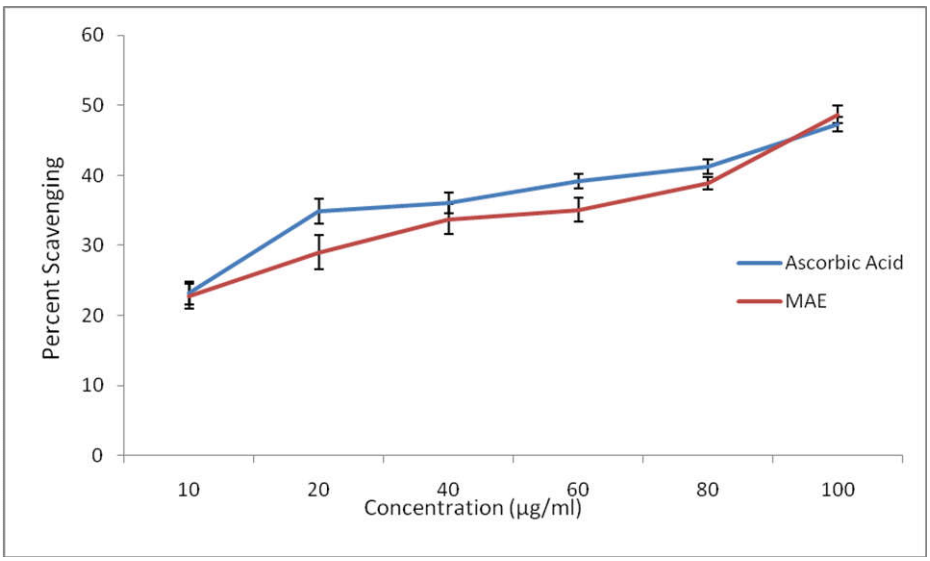

Figure 2: Percent Scavenging of MAE and Ascorbic acid in NO scavenging assay

Table 3: Nitric Oxide estimation in MAE treated macrophages rich cells

S. No.	Treatment groups	Concentration used (µg/ml)	Mean Total Nitric Oxide content (µM) ±SE
1	Control	**-**	59.56±0.867
2	Ascorbic acid	150	35.05±3.137
3	MAE	150	32.62±4.105
CD (5%)			**SE(m)±**
10.451			3.024

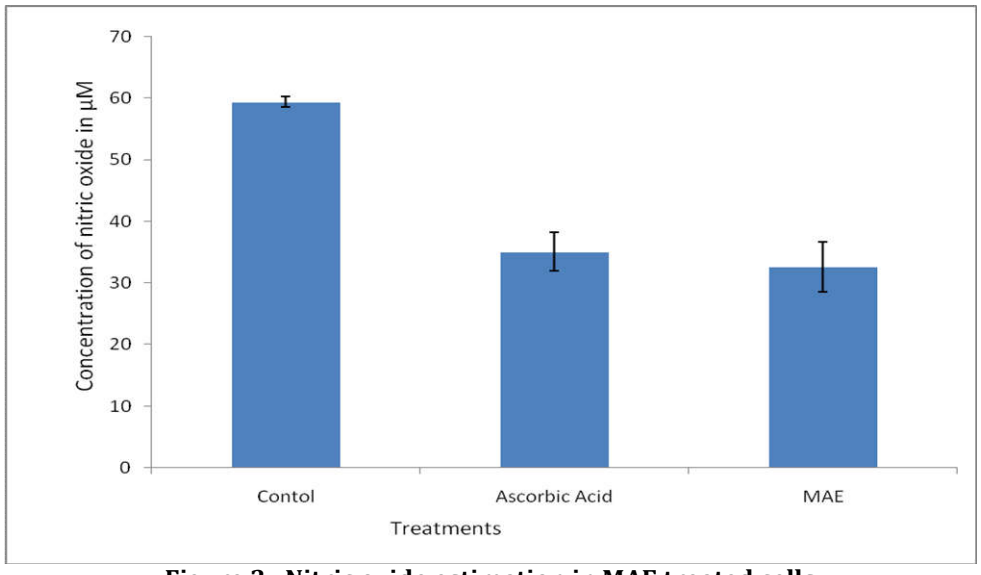

Figure 3: Nitric oxide estimation in MAE treated cells

Table 4: Lipid peroxidation in chicken lymphocytes treated with MAE

S. No.	Treatment groups	Concentration used (µg/ml)	Mean LPO nMmda/g ± SE
1.	Control	-	144.35±0.940
2.	Ascorbic acid	150	130.13±2.221
3.	MAE	150	123.37±4.951
CD (5%)			**SE(m)±**
15.226			4.406

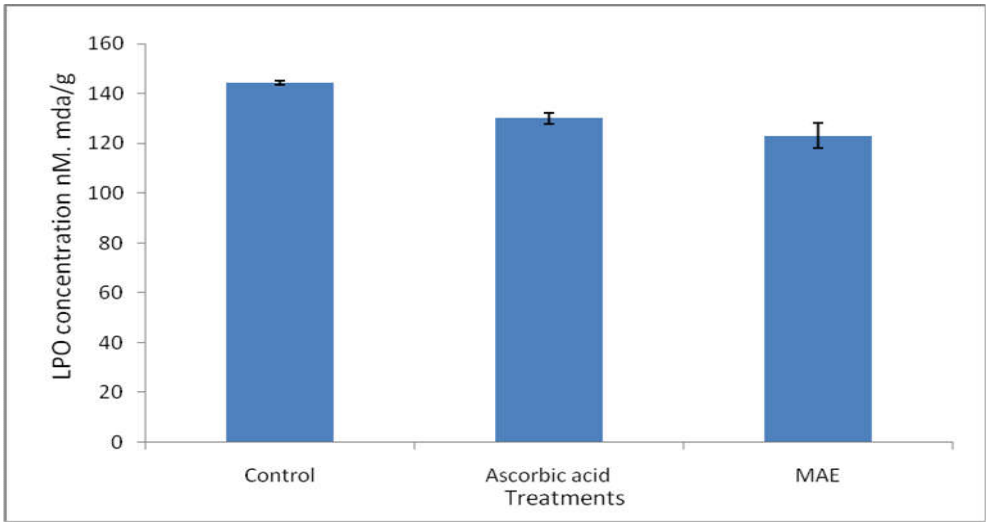

Figure 4: Lipid peroxidation in chicken lymphocytes treated with MAE

Table 5: Reduced glutathione Content in chicken lymphocytes cells treated with MAE

S. No.	Treatment groups	Concentration used (µg/ml)	Mean GSH content (mM/ml) ± SE
1	Control	-	3.92±0.056
2	Ascorbic acid	150	5.62±0.345
3	MAE	150	5.62±0.329
CD (5%)			SE(m)±
0.9595			0.2776

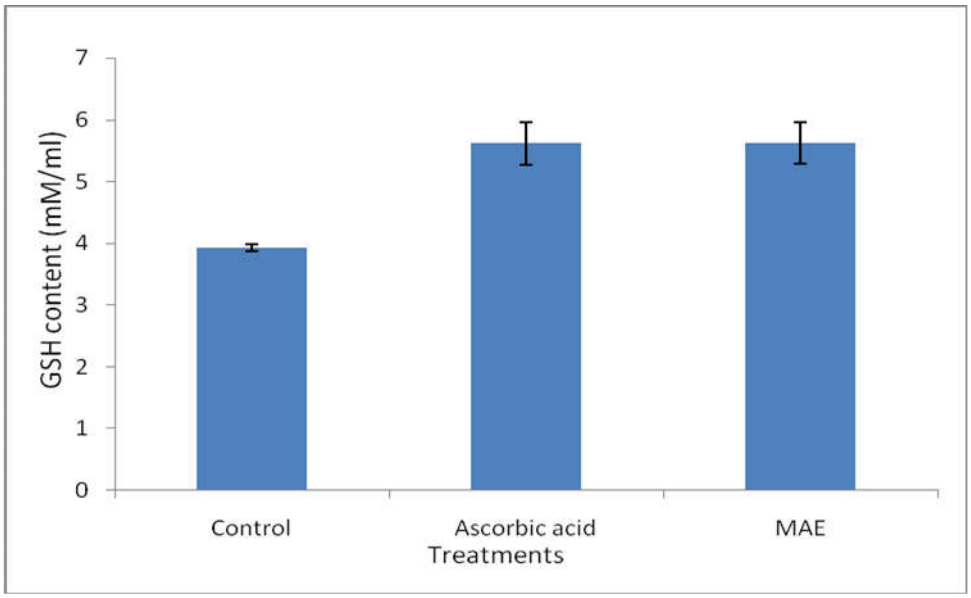

Figure 5: GSH content in chicken lymphocytes treated with MAE

Table 6: Superoxide Dismutase Content in chicken lymphocytes treated with MAE

S. No.	Treatment groups	Concentration used (µg/ml)	Mean SOD units/mg of protein ± SE
1	Control	-	66.52±0.202
2	Ascorbic acid	150	197.35±4.164
3	MAE	150	116.84±5.447
CD (5%)			**SE(m)±**
13.686			3.960

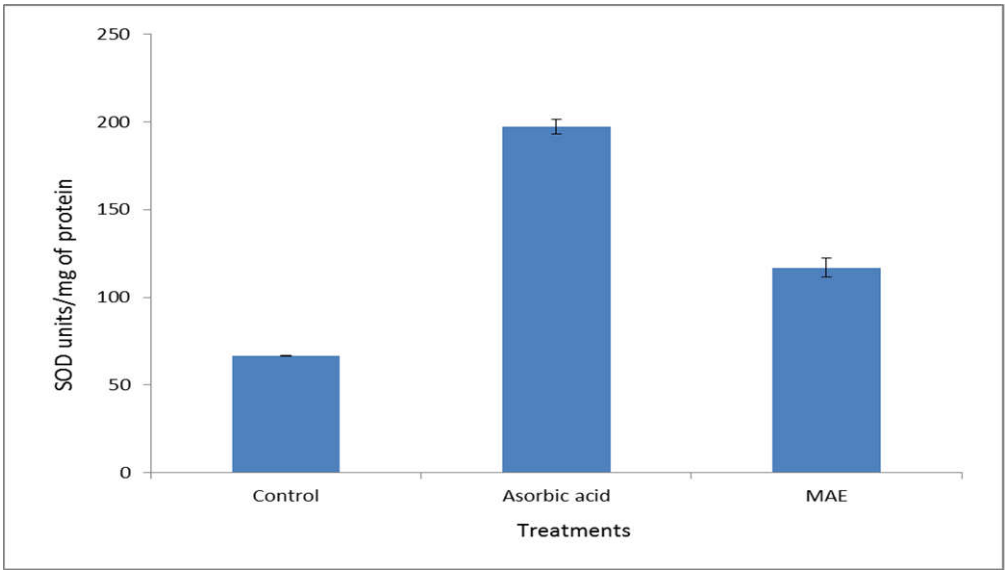

Figure 6: Superoxide Dismutase content in chicken lymphocytes treated with MAE

Table 7: Catalase activity in chicken lymphocytes cells treated with MAE

S. No.	Treatment groups	Concentration used (µg/ml)	Mean H_2O_2 utilized mM/min/mg of protein ± SE
1	Control	-	134.15±12.09
2	Ascorbic acid	150	223.97±4.075
3	MAE	150	185.99±3.054
CD (5%)			**SE(m)±**
29.593			8.563

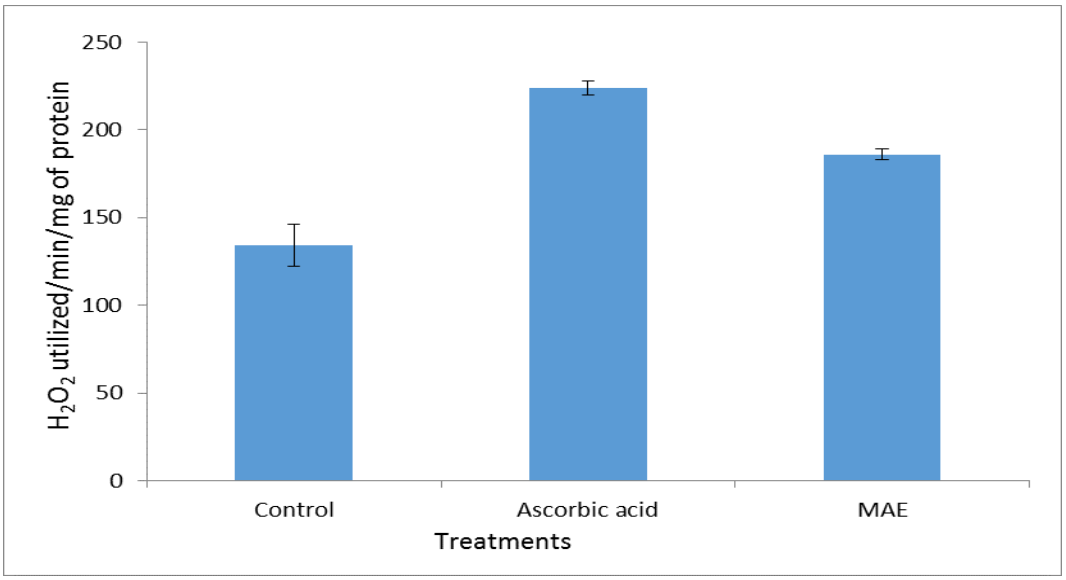

Figure 7: Catalase activity in chicken lymphocytes cells treated with MAE

CONCLUSION

Thus, from outcome of the present study it could be concluded that hydromethanolic leaf extract of *Melia azedarach* showed significant antioxidative potentially in terms of scavenging free radicals produced by various *in vitro* and cellular enzymatic antioxidants which was observed in MAE exposed chicken lymphocytes. *Melia azedarach* could be a potential source of natural plant antioxidants, however there is need for further explore this bioresource through various biochemical and molecular analyses.

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REFERENCES

1. Aebi, H. (1984). Catalase *in vitro*. In Methods in enzymology 105(2):121-126.
2. Ahmed, M. F., Ibrahim, M., Ahmed, M. A., Thayyil, H. & Zameeruddin K. (2008). Antioxidative activity of *Melia azedarach* Linn leaf extract. *Iranian Journal of Pharmacology and Therapeutics*, *7*(1):31-0.
3. Ahmed, M.F., Rao, A. S., Ahemad, S.R. & Ibrahim, M. (2012). Phytochemical Studies and Antioxidant Activity of Melia Azedarach Linnleaves by Dpph Scavenging Assay. International Journal of Pharmaceutical Applications, 3(1): 2012, Pp 271-276.
4. Ali, S. S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A. & Bora U. (2008). Indian medicinal herbs as sources of antioxidants. *Food Research International*, 41(1): 1-15.
5. Ambwani, S., Tandon, R., Ambwani, T.K. & Malik, Y.S. (2018). Current knowledge on nanodelivery systems and their beneficial applications in enhancing the efficacy of herbal drugs. Journal of Experimental Biology and Agricultural Science, 6(1): 87-107.
6. Andreadis A.A., Hazen S.L., Comhair S.A. and Erzurum S.C. (2003). Oxidative and nitrosative events in asthma. *Free Radical Biology and Medicine*, 35(3):213-225.
7. Deb, K., Kaur, A., Ambwani, S. & Ambwani, T.K. (2018). Preliminary phytochemical analyses of hydromethanolic leaf extract of *Melia azedarach* L. Journal of Medicinal Plants Studies, 6(3): 04-08.
8. Deb, K., Kaur, A., Ambwani, T. K. & Ambwani S. (2017). Assessment *of in vitro* immunosuppressive effects of *Melia azedarach* L. in chicken lymphocytes at National Symposium on Innovations in Horticulture: Production to Consumption held in GBPUA&T, Pantnagar on *September 14-15, 2017* p. 50
9. Ellman, G. L. (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 82(1): 70-77.
10. Hilton, J. W. (1989). Antioxidants, function, types and necessity of inclusion in pet foods. Canadian Veterinary Journal, 30(8):682-684.
11. Jagetia, G.C., Rao, S.K., Baliga, M.C. & Babu, K.S. (2004). The Evaluation of Nitric Oxide Scavenging Activity of Certain Herbal Formulations in vitro: A Preliminary Study. Phytotherapy Research. 18(4): 561–565.
12. Janossy, G. & Greaves, M. F. (1971). Lymphocyte activation: I. Response of T and B lymphocytes to phytomitogens. Clinical and Experimental Immunology, 9(4): 483.
13. Kalpana, K. B., Srinivasan, M. & Menon, V. P. (2009). Evaluation of antioxidant activity of hesperidin and its protective effect on H2O2 induced oxidative damage on pBR322 DNA and RBC cellular membrane. Molecular and cellular biochemistry, 323(1-2): 21-29.
14. Kumar, S, Sharma, S. & Jitpal, S. (2011). *In vivo* Antihyperglycemic and antioxidant potential of *Piper longum* fruit. J Pharm Res., 2011:4:1-4.
15. Madesh, M., Benard, O. & Balasubramanian, K. A. (1998). Glutathione modulates lipid composition of human colon derived HT-29 cells. The International Journal of Biochemistry & Cell Biology, 30(12): 1345-1352.
16. Marcocci, L., Packer, L., Droy-Lefaix, M. T., Sekaki, A. & Gardès-Albert, M. (1994). Antioxidant action of Ginkgo biloba extract EGb 761. *Methods in enzymology*, 234: 462-475.
17. Murphy, M. P. (1999). Nitric oxide and cell death. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1411(2-3): 401-414.
18. Nahak, G. & Sahu, R.K. (2010). In vitro antioxidative acitivity of Azadirachta indica and Melia azedarach Leaves by DPPH scavenging assay Nature and Science, 8(4):22-28.
19. Ohkawa, H., Ohishi, N. & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2): 351-358.
20. Pokorny, J. (2007). Are natural antioxidants better and safer than synthetic antioxidants? European Journal of Lipid Science and Technology, 109(6):629-642.
21. Roopashree, T.S., Dang, R., Rani, R.H.S. & Narendra, C. (2008). Antibacterial activity of antipsoriatic herb: Cassia tora, Momordica charantia and Calendula officinalis. *International* Journal of Applied Research in Natural Products, 1(3): 20-28.
22. Rose, N. R. and Friedman, H. (1976). Manual of clinical immunology. Manual of Clinical Immunology.
23. Sharma, D. & Paul, Y. (2013). Preliminary and pharmacological profile of *Melia azedarach* L.: an overview. Journal of Applied Pharmaceutical Science, 3(12): 133-138.

24. Shivhare, Y., Singour, P. K., Patil, U. K. & Pawar, R. S. (2010). Wound healing potential of methanolic extract of Trichosanthes dioica Roxb (fruits) in rats. Journal of Ethnopharmacology, 127(3): 614-619.
25. Sies, H., Koch, O. R., Martino, E. & Boveris, A. (1979). Increased biliary glutathione disulfide release in chronically ethanol-treated rats. FEBS letters, 103(2): 287-290.
26. Stuehr, D. J. & Nathan, C. F. (1989). Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. Journal of Experimental Medicine, 169(5): 1543-1555.
27. Surai, P. F. (2016). Antioxidant systems in poultry biology: superoxide dismutase. Journal of Animal Research and Nutrition, 1(1): 1-17.
28. Thakur, A.V., Ambwani, S., Ambwani, T.K., Ahmad, A.H. & Rawat, D.S. (2018). Evaluation of phytochemicals in the leaf extract of *Clitoria ternatea* Willd. through GC-MS analysis. Tropical Plant Research 5(2): 200–206
29. Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactionst, 160 (1): 1-40.
30. Wolfe, K., Xianzhong, W. U. & Liu, R. H. (2003). Antioxidant activity of apple peels. Journal of Agricultural Food Chemistry, 51:609–614.

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