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INVITRO ANTIOXIDANT CAPACITY AND FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS ANTI DEPRESSANT DRUGS

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ABSTRACT

One of the major cause of depression and propagation of depression is oxidative stress and this leads to further complications like Parkinson's disease, Alzheimer's disease and other neurodegenerative conditions. Current study involved different reuptake inhibitors used in combating depression and the antioxidant activity. DPPH radical scavenging assay, Nitric oxide radical scavenging assay, FRAP, Phosphomolybdenum assay and assessment of inhibition of lipid peroxidation- these five methods were adopted for investigation of the antioxidant activity of Modafinil, Fluoxetine, Duloxetine and Vilazodone. This study indicated Modafinil as best DPPH radical scavenger, vilazodone as the best scavenger of nitric oxide radicals, duloxetine as the drugs with best reducing potential and fluoxetine as the best drug to inhibit lipid peroxidation amongst the four drugs. These findings suggest that these reuptake inhibitors have the radical scavenging capacity which may show beneficial effect in controlling depression besides their established pharmacological profile.

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INTRODUCTION

Sadness is a normal reaction to difficult times we generally go through. Depression is one of the most common mental disorders that presents with loss of interest or pleasure, feelings of guilt or low self-worth, depressed mood, disturbed sleep or appetite, poor concentration and low energy. [1,2] The patient suffering from depression may also face trouble in making decision, perception, memory retrieval, memorizing things. These symptoms caused by depression vary from person to person depending upon inherited traits, age, gender and cultural background. Sometimes depression can lead to suicide. [3,4]The main symptoms of depression are because of the functional deficiency of the brain monoaminergic transmitter, Norepinephrine (NE), Dopamine (DO) and/or serotonin (5-hydroxytryptamine). [5,6]

Oxidative stress occurs when the generation of free radicals and active intermediates in a system exceeds the system's ability to neutralize and eliminate them and/or when there is an increase in free radicals (FR) or decrease in anti-oxidants or an imbalance between them in our cells. [7,8] Under physiological condition in a living organism the reactive oxygen intermediate (ROI) and the reactive nitrogen intermediate (RNI) is constantly being produced. [9] The Reactive oxygen intermediate (ROI) are superoxide radical ($0_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), singlet oxygen ($^1O_2$) and many other. The reactive nitrogen intermediate (RNI) includes nitric oxide ($\cdot NO$) including few other. Hence, (ROS) is collective term used for a group of oxidant, these ROS includes free radicals or molecular species capable of generating free radicals. [10-11] During various pathological conditions their production increases by several folds. The ROS regulate many metabolic and cellular processes including, gene expression, immunity, migration, proliferation and wound healing.

Many process such as apoptosis, viral proliferation and inflammatory reaction can be influenced by oxidative stress. [12] In these biological processes, gene transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) act as oxidative stress sensor through their own oxidation and reduction cycling. Oxidative stress has been involved in several diseases including cancer, malaria, atherosclerosis, chronic fatigue syndrome, and rheumatoid arthritis and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease. [13,14] Neurodegenerative diseases causes progressive loss of specific neuronal cell population which is responsible for dysfunction or death of neuronal cell that contributes to disease pathogenesis. Antidepressant constitute a heterogeneous group of compound function by different mechanism of action including serotonin-noradrenaline (norepinephrine) reuptake inhibitors (SNRIs) like Duloxetine, serotonin antagonist and reuptake inhibitors, selective serotonin reuptake inhibitors (SSRIs) like Fluoxetine, noradrenaline reuptake inhibitor (NRIs), noradrenaline-dopamine reuptake inhibitors (NDRIs) like Modafinil, nonadrenaline-dopamine releasing agent (NDRAs) tricyclic antidepressant (TCAs) and monoamine oxidase inhibitors (MAOIs). [15,16]

This project aimed to determine the antioxidant property of various antidepressant reuptake inhibitors like fluoxetine, duloxetine, modafinil and vilazodone. This was done by evaluating the free radical scavenging activity of above mentioned antidepressant drugs using five different *in vitro* methods namely DPPH based free radical scavenging activity, nitric oxide radical scavenging assay, Ferric reducing potential assay (FRAP), assessment of inhibition of lipid peroxidation and phosphomolybdenum assay.

MATERIALS AND METHODS

MATERIALS

All the chemicals used were analytical grade. L-ascorbic acid [CAS Number-50-81-7] was purchased from SDFCL, Mumbai, India.2,2-diphenyl-1-picrylhydrazyl (DPPH) [CAS Number-1898-66-4] was purchased from Himedia laboratories, Mumbai, India. Methanol [CAS Number-67-56-1] was purchased from Rankem, India. Dimethyl sulfoxide (DMSO) [CAS Number-67-68-5] was purchased from Fischer Scientific, Mumbai, India. Sulphanilamide [CAS Number-63-74-1] was purchased from Spectrochem, Mumbai. N-(1-naphthyl)-ethyl-enediamine dihydrochloride [CAS Number-1465-25-4] was purchased from SDFCL, Mumbai, India. Orthophosphoric acid [CAS Number 7664-38-2] was purchased from Merck, India. Sodium nitroprusside [CAS Number-13755-38-9] was purchased from Merck, India. Ethanol [CAS Number-64-17-5] was purchased from Rankem, India. Hydrochloric Acid [CAS Number -7647-01-0] was purchased from Rankem, India. Potassium ferrocyanide [CAS Number-14459-95-1] was purchased from Merck, Mumbai. Ferric chloride [CAS Number – 7705-08-0] was purchased from Rankem, India. Sodium Dodecyl sulphate [CAS Number – 151-21-3] was purchased from Merck, Mumbai. Ammonium molybdate [CAS Number – 12054-85-2] was purchased from Spectrochem, India. Disodium hydrogen phosphate dihydrate [CAS Number – 10028-24-7] was purchased from Rankem, India. Sulphuric Acid [CAS Number – 7664-93-9] was purchased from Fischer, Scientific, Mumbai, India. Ferrous Sulphate heptahydrate [CAS Number – 7782-63-0] was purchased from Rankem, India. Glacial acetic acid [CAS Number – 64-19-7] was purchased from Merck, Mumbai, India. Trichloroacetic Acid [CAS Number – 76-03-9] was purchased from Merck, Mumbai, India. Thiobarbituric Acid [CAS Number- 504-17-6] was purchased from Spectrochem, Mumbai, India. Butan-1-ol [CAS Number – 71-36-3] was purchased from Fischer Scientific, Mumbai, India.

METHODOLOGY

Determination of DPPH Scavenging Assay:

This method was adopted with slight modification. The DPPH scavenging assay was performed for all the 4 drugs at concentration (50, 100, 200, 400 and 800 µg/ml). The drugs were solubilized in suitable solvents with vortexing for sufficient time to solubilize. 50µL of the drug solutions of each of five different concentrations were taken in a test tube followed by addition of methanol q.s to 3ml. 150µL of 0.13% w/v solution of DPPH in methanol was then added in each test tubes and left for sometimes. The absorbance of the test sample was measured with UV Spectrophotometer (Schimadzu UV-1800) at 517 nm. The decrease in absorbance upon increasing drug concentration implicates more scavenging of free radicals. [17] The percentage (%) inhibition was calculated from the formula-

$$\% \text{ inhibition} = \frac{Abs\ of\ control - Abs\ of\ test}{Abs\ of\ Control} \times 100$$

Determination of Nitric Oxide (NO) Scavenging Assay:

The NO scavenging assay was performed for all of the 4 drugs at concentration 50-800µg/ml. Scavenging of the nitrite ions was assessed with Griess reagent (1% w/v sulphanilamide, 5% w/v phosphoric acid, 0.1% w/v N-(1-naphthyl)-ethyl-enediamine dihydrochloride). 50µl of the stock solution was pipetted out in test tube. 1ml of 3 hours incubated Sodium Nitropruside(SNP) was added to each test tube ranging from 50-800µg/ml, followed by addition of 0.5ml of above prepared Griess reagent. The diazotization of nitrite ions with sulphanilamide and subsequent coupling reaction with N-(1-naphthyl)-ethyl-enediamine dihydrochloride generates a pink chromophore absorbance which was measured at 596 nm.The antioxidant property of the drugs were analysed taking ascorbic acid as standard. [18]

Ferric ion reduction by assay of ferric reducing power (FRAP):

In this method Fe^{3+}ion is transformed into Fe^{2+} ion by donating an electron in presence of antioxidant compound. 0.5ml of deionized water is added in each of the test solution ranging from 50-800µg/ml concentration. Further 0.09ml of 95% ethanol, 0.15ml each of 1M HCl and 1% potassium ferrocyanide was added to it. After this 0.05ml 1% Sodium dodecyl sulphate and 0.1ml 0.2% $FeCl_3$was added into it. A Prussian blue colour appeared after addition of all of the above mentioned chemicals in appropriate amount. All the test tube were vortexed for 20 min for the uniform distribution of colour throughout the sample solution. Finally readings were taken at 700nm in a UV visible spectrophotometer (Schimadzu UV 1800). The antioxidant properties of the drugs were analysed taking ascorbic acid as standard. [18]

Assessment of inhibition of lipid peroxidation(LPO):

In this method the extent of lipid peroxides formed was assayed by a modified version of Thiobarbituric Acid Reactive Substances (TBARS) using egg yolk homogenate as lipid-rich media. Yolk of egg was homogenated for 10 minutes. 0.5ml of 10% homogenated egg was added to 100µl of the drug solution of different concentration (50, 100, 200, 400 and 800 µg/ml) of each of the four drugs. The volume was made upto 1ml with distilled water. 0.05ml of 0.07M $FeSO_4$ was added to the above mixture and further incubated for 30 min, to enhance for lipid oxidation. Then 1.5ml of 0.8% w/v thiobarbituric acid (TBA) prepared in 1.1%w/v Sodium Dodecyl Sulphate, 1.5ml of 20% Acetic Acid and 0.05ml of 20%w/v thiochloroacetic acid (TCA) were sequentially added. The resulting mixture was heated at 95^0 C for 1 hours. Then it was cooled at room temperature. After cooling 5ml of Butan-1-ol was added to each of the test solution and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer was collected and the absorbance of which was measured at 532 nm. [19]

Phosphomolybdenum assay:

This method slightly modified in which Phosphate-Mo (vi) is reduced to Phosphate-Mo (v), giving bluish green color which was measured by its absorbance. 1.8ml of distilled water was added in the test tube containing varying concentration (50-800µg/ml) of the drug. Then 2 ml of freshly prepared phosphomolybdenum reagent was added to each of the test tube. The tubes were labeled and then kept on water bath at 95˚C for 15 min. The mixtures were cooled down to room temperature and readings were taken at 695nm in the same spectrophotometer. The antioxidant activity of the drugs was evaluated taking ascorbic acid as standard. [20]

RESULTS AND DISCUSSION

RESULTS

Determination of DPPH Radical Scavenging Assay:

The extent of DPPH radicals scavenging at 800µg/ml by ascorbic acid (figure 1), fluoxetine, duloxetine, Modafinil and vilazodone was found to be 97.87%, 33.88 ± 0.0900^c, 29.22 ± 0.0900^c, 42.06 ± 0.0450^c and 37.66 ± 0.1800^c respectively (figure 3).The IC_{50} value shown by these accordingly drugs were 117.4±0.02,9724 ± 38.5^C, 4516 ± 5.5^C, 2716 ± 45039^C and 4223 ± 3^C respectively. By analyzing the data it was confirmed that Modafinil has better antioxidant property that other mentioned drugs.

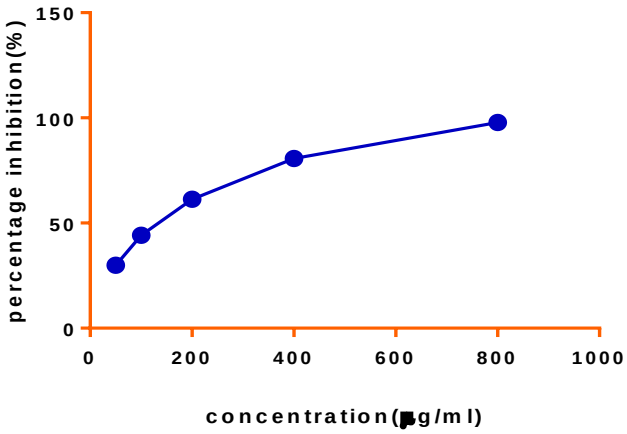

Figure1: Standard curve of ascorbic acid taking 50,100,200,400,800 µg/ml concentrations by DPPH method.

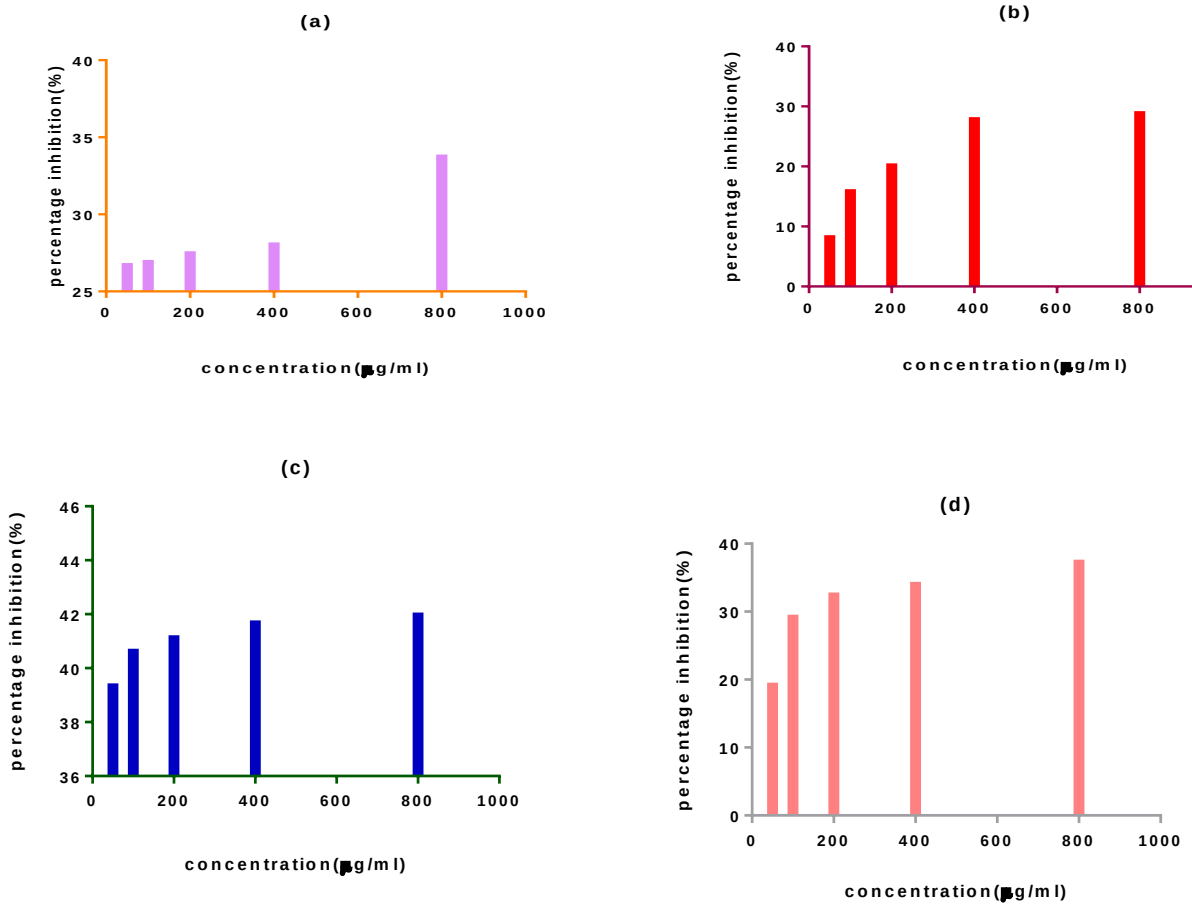

Figure 2: Shows antioxidant potential by plotting % inhibition versus different concentration (50,100,200,400,800 µg/ml) of different antidepressant drugs (a) Fluoxetine (b) Duloxetine (c) Modafinil (d) Vilazodone by DPPH method.

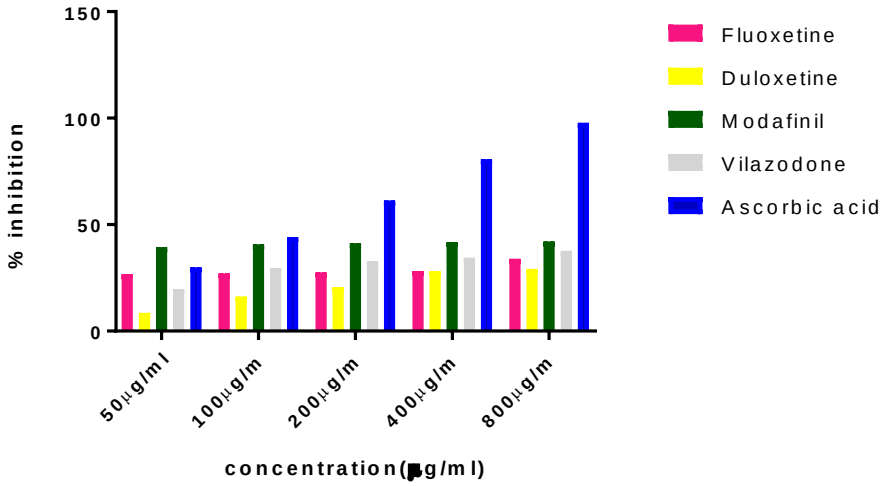

Figure 3: Antioxidant Potential of all drugs and standard by DPPH method.

Determination of NO Radical Scavenging Assay:

A dose dependent nitric oxide radical scavenging activity is established taking ascorbic acid as antioxidant (figure 4). At 800µg/ml, the extent of NO radical scavenging by ascorbic acid, fluoxetine, duloxetine, Modafinil and vilazodone was 36.81%, 38.15 ± 0.1000[b], 35.11 ± 0.0900[a] , 61.22 ± 0.1800[c]and 86.78 ± 0.2250[c] respectively(figure-6).The IC_{50} value of ascorbic acid, fluoxetine, duloxetine , Modafinil and vilazodone were 2650±49.5[c], 2237 ± 36.5[a], 2661 ± 5[a], 227 ± 3[c]and 11.97 ± 0.03[c]. The lowest value of IC_{50} of vilazodone proves that it has best scavenging potential than other drugs including ascorbic acid.

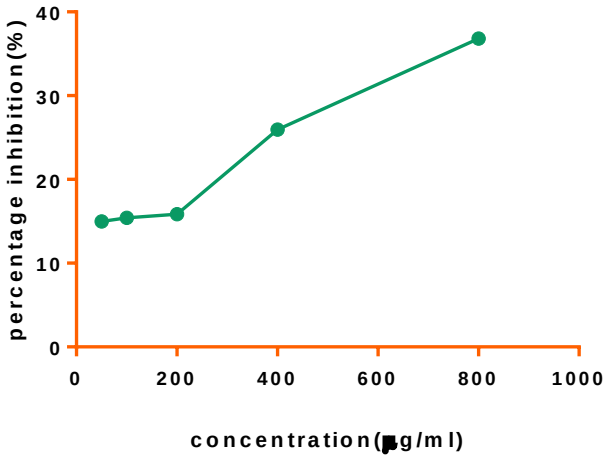

Figure 4: Standard curve of ascorbic acid taking 50,100,200,400,800 µg/ml concentrations by NO method.

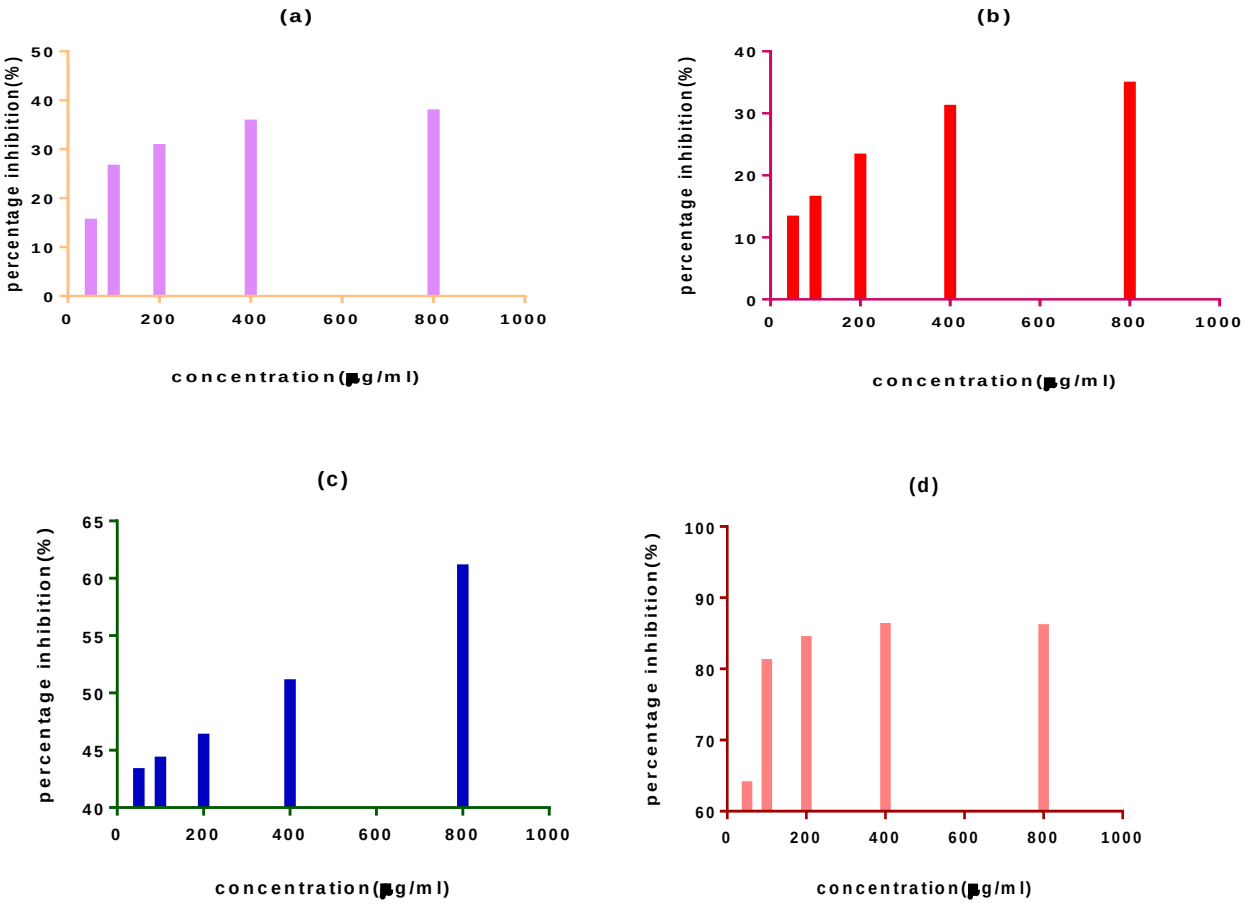

Figure 5: Shows antioxidant potential by plotting % inhibition versus different concentration (50,100,200,400,800 µg/ml) of different antidepressant drugs (a) Fluoxetine (b) Duloxetine (c) Modafinil (d)Vilazodone by NO method.

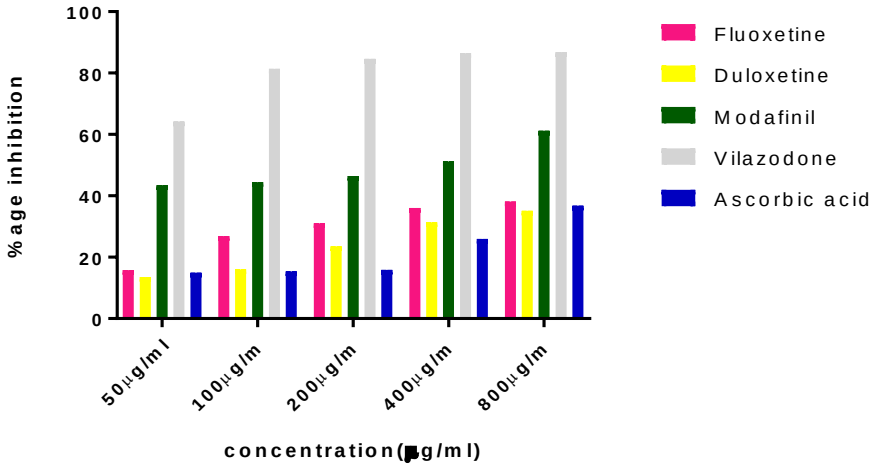

Figure 6: Antioxidant Potential of all drugs and standard by NO method.

Determination of FRAP Radical Scavenging Assay:

The dose dependent increase in radical scavenging activity of ascorbic acid measured by FRAP process is shown in (figure-7). The scavenging capacity of all drugs viz., ascorbic acid, fluoxetine, duloxetine, Modafinil and vilazodone at 800µg/ml was calculated to be 98.47%,96.38 ± 0.0450^{b}, 93.33 ± 0.2700b, 40.05 ± 0.4050^{c} and 83.50 ± 0.0450^{c} respectively(figure-9). IC_{50} value of ascorbic acid, fluoxetine, duloxetine, Modafinil and vilazodone were 271.2±0.06, 80.67 ± 0.67^{c}, 80.26 ± 0.255^{c}, 6828 ± 4.5^{c} and 109 ± 0.95^{c}.The ferric ion reduction capacity of fluoxetine and duloxetine obtained was almost same. So both of these two drugs have better and equivalent scavenging potential.

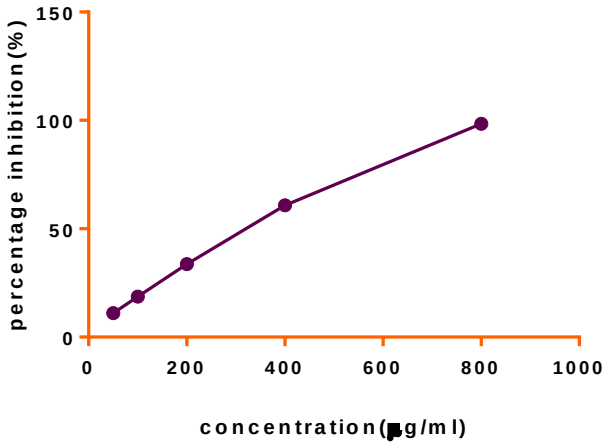

Figure 7: Standard curve of ascorbic acid taking 50,100,200,400,800 µg/ml concentrations by FRAP method.

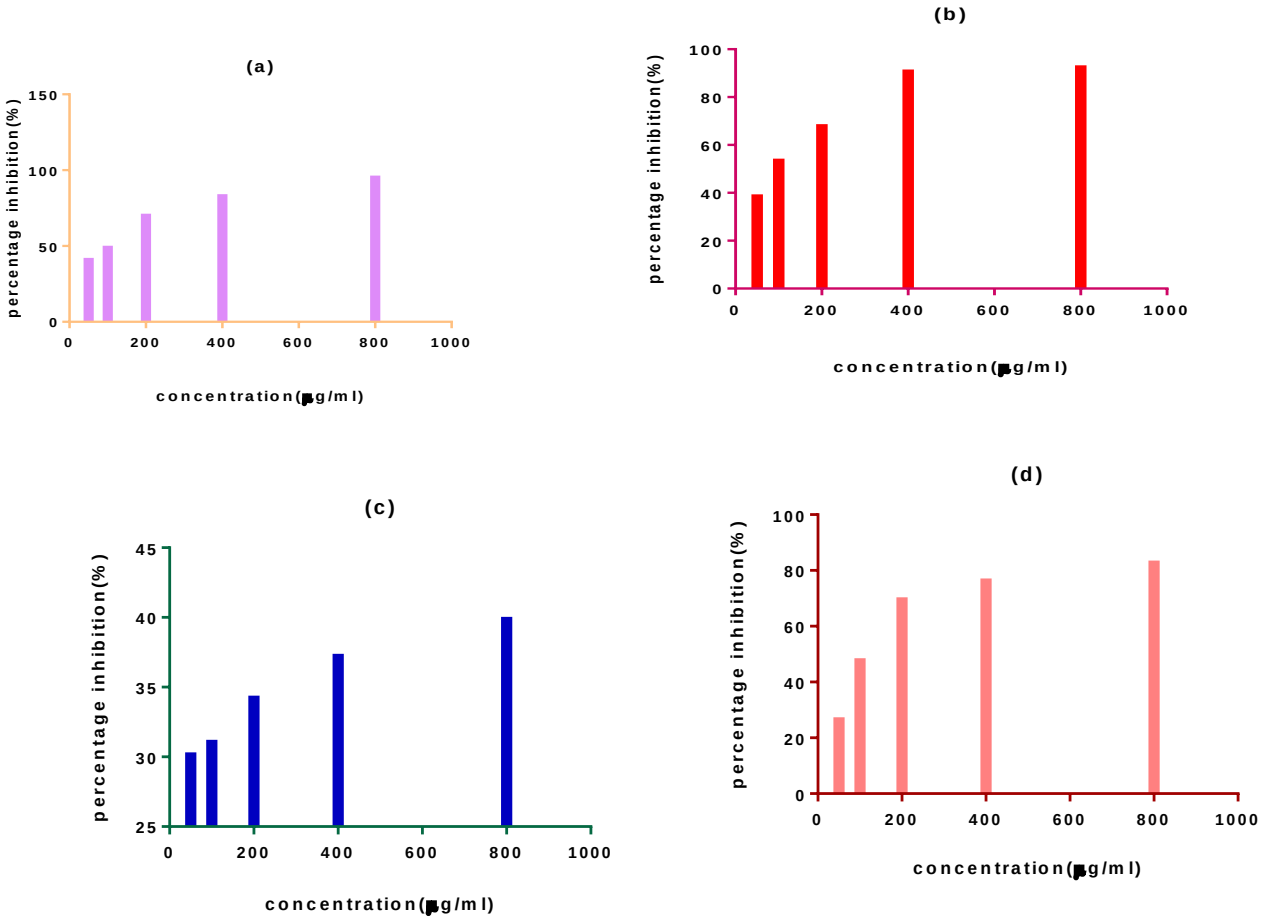

Figure 8: Shows antioxidant potential by plotting % inhibition versus different concentration (50,100,200,400,800 µg/ml) of different antidepressant drugs (a) Fluoxetine (b) Duloxetine (c) Modafinil (d) Vilazodone by FRAP method.

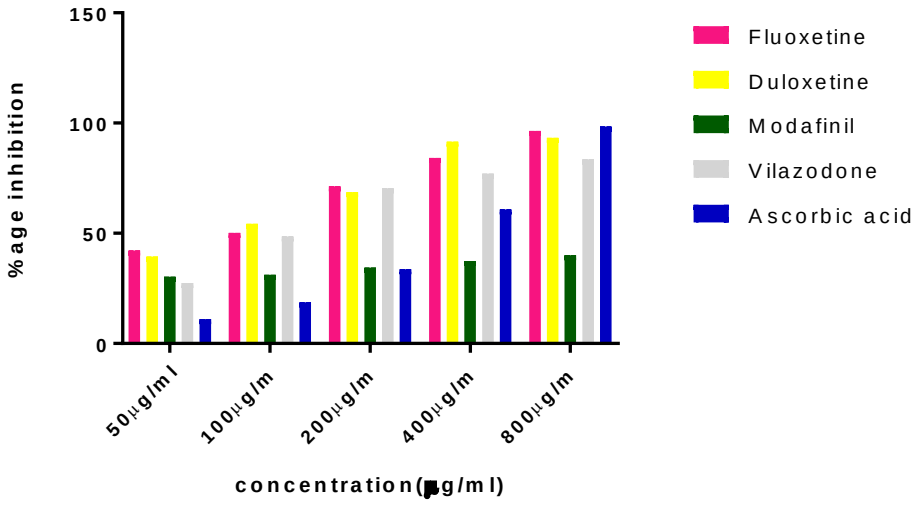

Figure 9: Antioxidant Potential of all drugs and standard by FRAP method.

Determination of LPO Radical Scavenging Assay:

The dose dependent increase in radical scavenging activity of ascorbic acid measured by FRAP process is shown in (figure-10). At 800µg/ml, the extent of inhibition of lipid peroxidation by ascorbic acid, fluoxetine, duloxetine, Modafinil and vilzodone obtained were 98.31%,71.35±0.2000^{c}, 47.87±0.1000^{c}, 36.74±0.1550^{c} and 57.25± 0.1500^{c}respectively(figure-11).The IC_{50} data of ascorbic acid and different drugs in same sequence was found to be 141.8±0.09, 151 ± 0.05^{b}, 1665 ± 4.5^{c}, 1777 ± 9^{c} and 640.9 ± 0.9^{c}. These data clearly indicates fluoxetine has better potential to inhibit lipid peroxidation.

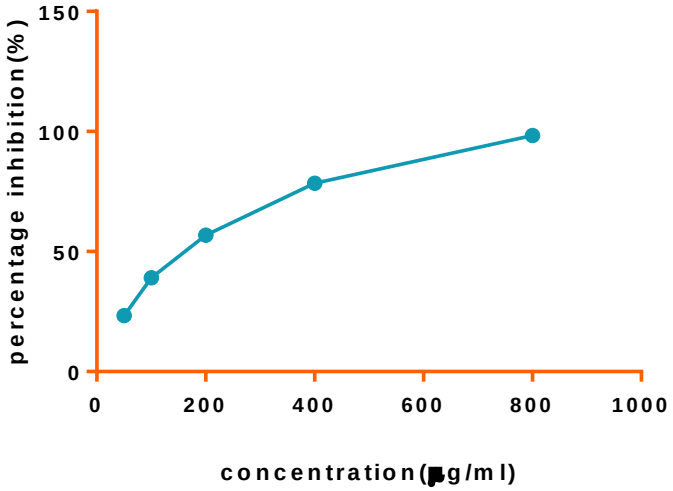

Figure 10: Standard curve of ascorbic acid taking 50,100,200,400,800 µg/ml concentrations for LPO method.

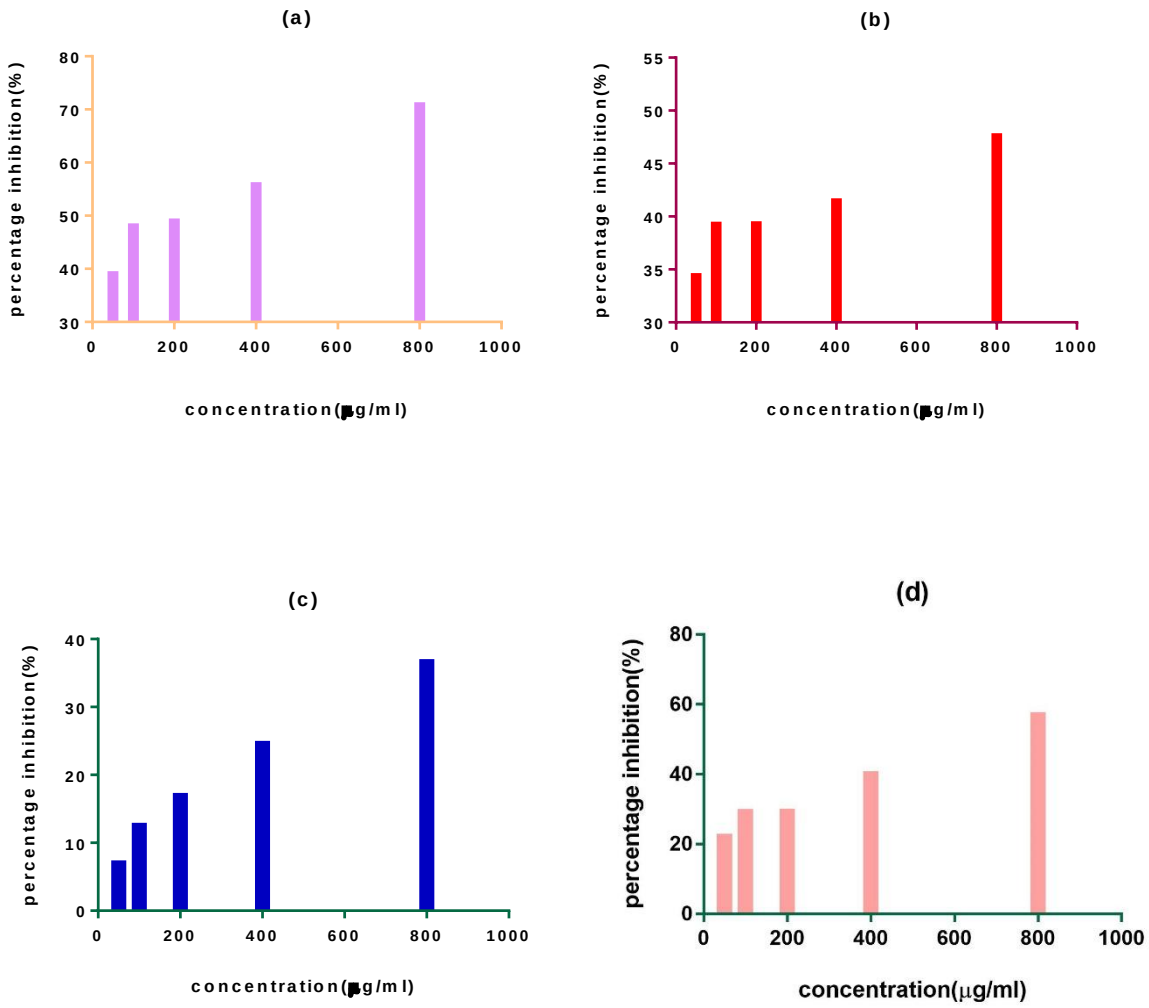

Figure 11: Shows antioxidant potential by plotting % inhibition versus different concentration (50,100,200,400,800 µg/ml) of different antidepressant drugs (a) Fluoxetine (b) Duloxetine (c) Modafinil (d) Vilazodone by LPO method.

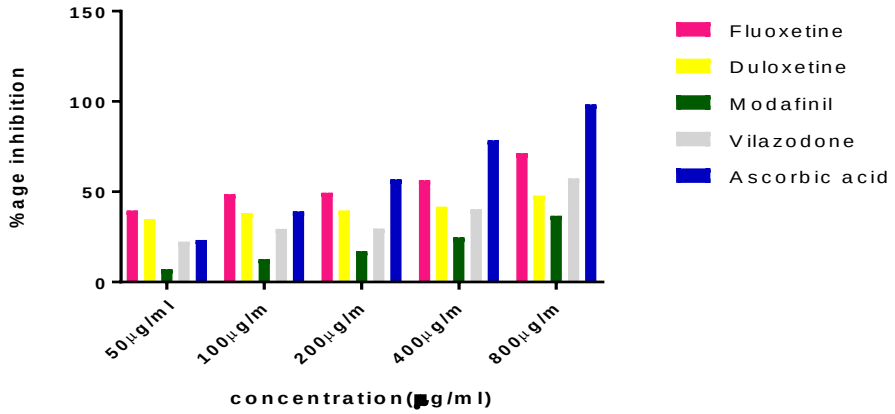

Figure 12: Antioxidant Potential of all drugs and standard LPO method.

Determination of Phosphomolybdenum Radical Scavenging Assay:

A dose dependent increase in antioxidant activity of ascorbic acid was found when the extent of antioxidant activity was measured by phosphomolybdenum assay process (figure-13).The radical scavenging strength of ascorbic acid, fluoxetine, duloxetine, Modafinil and vilazodone at 800µg/ml were obtained as 67.39%, 34.61 ± 0.04500^{c}, 63.22 ± 0.1800^{b}, 27.17 ± 0.04500^{c} and 23.44 ± 0.0900^{c} respectively (figure-15). The IC_{50} of the respective drugs in same above sequence was 174.3±0.04, 2802 ± 2^{c}, 239.6 ± 0.45^{c}, 5650 ± 13.5^{c} and 3748 ± 5.5^{c}. By analyzing these data the duloxetine drug is predicted to be the best drug among all.

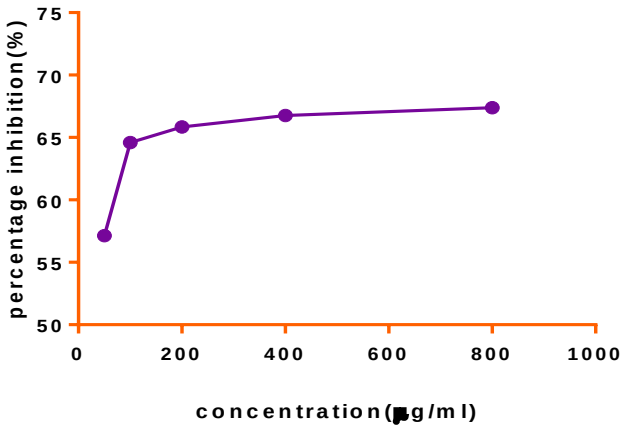

Figure 13: Standard curve of ascorbic acid taking 50,100,200,400,800 µg/ml concentrations by Phosphomolybdenum.

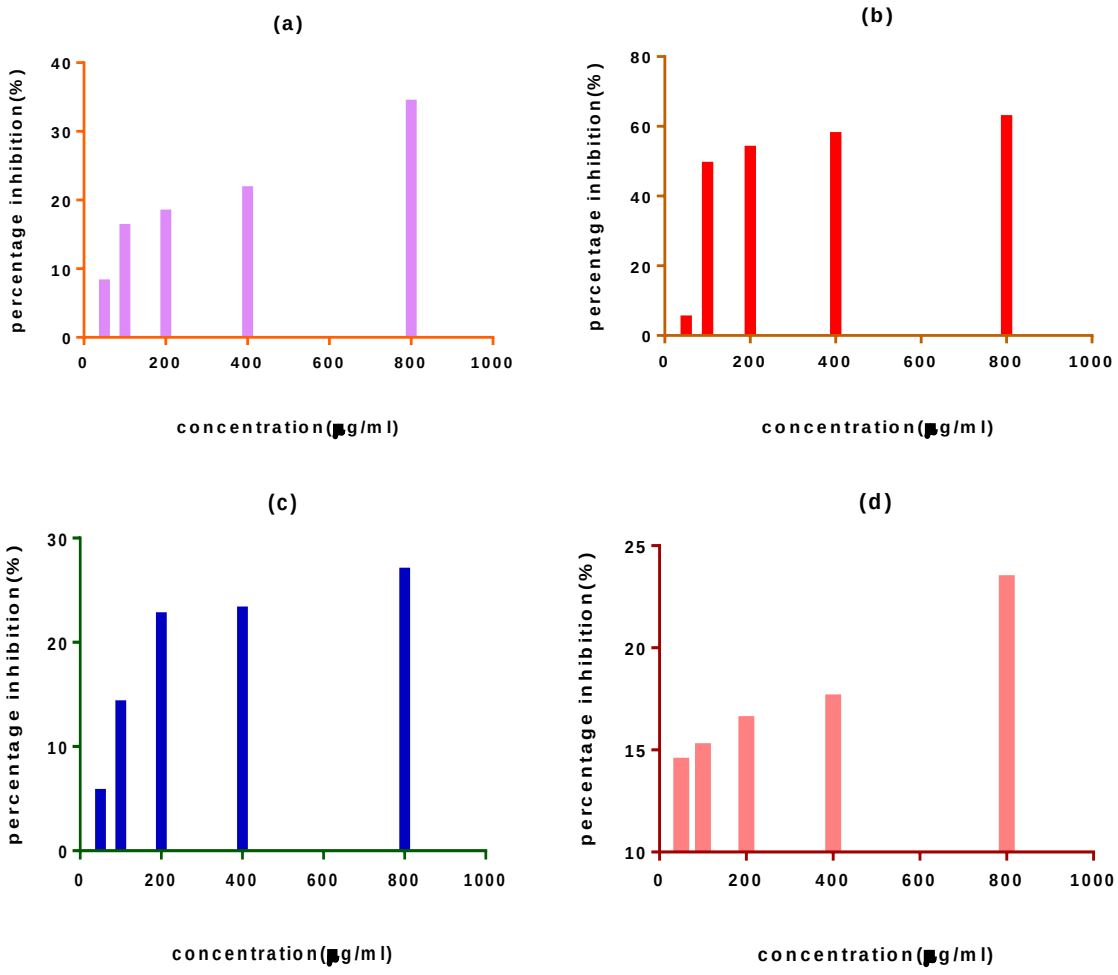

Figure 14: Shows antioxidant potential by plotting % inhibition versus different concentration (50, 100, 200, 400, 800 µg/ml) of different antidepressant drugs (a) Fluoxetine (b) Duloxetine (c) Modafinil (d) Vilazodone by Phosphomolybdenum method.

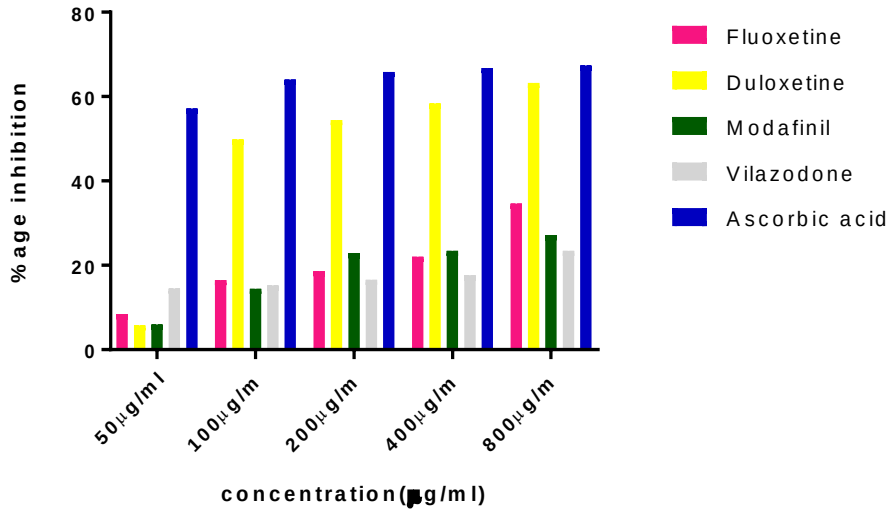

Figure 15: Antioxidant Potential of all drugs and standard Phosphomolybdenum method.

Statistical Analysis:

All samples were analyzed in triplicate and data are reported as Mean ± SEM. Statistical analysis was done with Graph Pad version 7. IC_{50} values were calculated taking concentration versus normalized response with variable slope in nonlinear regression.

Tabular Representation of Data:

Table No: 1: Free radical scavenging potential of Fluoxetine as % inhibition.

SL. No.	Concentration (µg/ml)	DPPH method	NO method	FRAP method	LPO method	Phosphomolybdenum Method
1	50	26.84 ± 0.1000^{b}	15.80 ± 0.1000^{a}	42.20 ± 0.1400^{c}	39.55 ± 0.1800^{c}	8.44 ± 0.3600^{c}
2	100	27.04 ± 0.4000^{c}	26.86 ± 0.1000^{c}	50.21 ± 0.2100^{c}	48.55 ± 0.1800^{c}	16.50 ± 0.3150^{c}
3	200	27.61 ± 0.3150^{c}	31.05 ± 0.0550^{c}	71.28 ± 0.0450^{c}	49.49 ± 0.3100^{b}	18.61 ± 0.2250^{c}
4	400	28.18 ± 0.1000^{c}	36.07 ± 0.1000^{c}	84.21 ± 0.1250^{c}	56.33 ± 0.0900^{c}	22.03 ± 0.0300^{c}
5	800	33.88 ± 0.0900^{c}	38.15 ± 0.1000^{b}	96.38 ± 0.0450^{b}	71.35 ± 0.2000^{c}	34.61 ± 0.04500^{c}

*Data are expressed as mean ± standard deviation (N=6), mean in the same column with different subscripts are significantly different by t-test for unpaired comparison at a-p < 0.05, b-p < 0.01, c-p < 0.001, ns= non-significant, p>0.05.

Table No: 2: Free radical scavenging potential of Duloxetine as % inhibition.

Sl. No.	Concentration (µg/ml)	DPPH method	NO method	FRAP method	LPO method	Phosphomolybdenum Method
1	50	08.55 ± 0.3600^{c}	13.50 ± 0.1350^{a}	39.38 ± 0.1000^{c}	34.66 ± 0.0900^{c}	5.77 ± 0.1800^{c}
2	100	16.22 ± 0.1300^{c}	16.70 ± 0.1200^{a}	54.26 ± 0.1000^{c}	38.13 ± 0.1300^{a}	49.83 ± 0.1350^{c}
3	200	20.50 ± 0.0450^{c}	23.50 ± 0.2250^{b}	68.72 ± 0.1350^{c}	39.55 ± 0.1800^{c}	54.44 ± 0.09000^{c}
4	400	28.22 ± 0.1800^{c}	31.39 ± 0.0450^{b}	91.50 ± 0.2250^{c}	41.72 ± 0.2250^{c}	58.39 ± 0.3150^{b}
5	800	29.22 ± 0.0900^{c}	35.11 ± 0.0900^{a}	93.33 ± 0.2700^{b}	47.87 ± 0.1000^{c}	63.22 ± 0.1800^{b}

*Data are expressed as mean ± standard deviation (N=6), mean in the same column with different subscripts are significantly different by t-test for unpaired comparison at a-p < 0.05, b-p < 0.01, c-p < 0.001, ns= non-significant, p>0.05.

Table No: 3: Free radical scavenging potential of Modafinil as % inhibition.

SL. No.	Concentration (µg/ml)	DPPH method	NO method	FRAP method	LPO method	Phosphomolybdenum Method
1	50	39.44 ± 0.8000^{c}	43.44 ± 0.0900^{c}	30.33 ± 0.1800^{c}	07.11 ± 0.0900^{c}	5.94 ± 0.06000^{c}
2	100	40.72 ± 0.4500^{b}	44.44 ± 0.0900^{c}	31.22 ± 0.1800^{c}	12.66 ± 0.1800^{c}	14.44 ± 0.2700^{c}
3	200	41.22 ± 0.1800^{c}	46.44 ± 0.2700^{c}	34.39 ± 0.0450^{c}	17.06 ± 0.0450^{c}	22.88 ± 0.09000^{c}
4	400	41.77 ± 0.0900^{c}	51.20 ± 0.1000^{c}	37.39 ± 0.0450^{c}	24.72 ± 0.0450^{c}	23.44 ± 0.09000^{c}
5	800	42.06 ± 0.0450^{c}	61.22 ± 0.1800^{c}	40.05 ± 0.4050^{c}	36.74 ± 0.1550^{c}	27.17 ± 0.04500^{c}

*Data are expressed as mean ± standard deviation (N=6), mean in the same column with different subscripts are significantly different by t-test for unpaired comparison at a-p < 0.05, b-p < 0.01, c-p < 0.001, ns= non-significant, p>0.05.

Table No: 4: Free radical scavenging potential of Vilazodon as % inhibition.

SL. No.	Concentration (µg/ml)	DPPH method	NO method	FRAP method	LPO method	Phosphomolybdenum Method
1	50	19.55 ± 0.0900^{c}	64.22 ± 0.1800^{c}	27.39 ± 0.0450^{c}	22.36 ± 0.0650^{a}	14.50 ± 0.2000^{c}
2	100	29.55 ± 0.1800^{c}	81.38 ± 0.0450^{c}	48.55 ± 0.1800^{c}	29.44 ± 0.3600^{b}	15.22 ± 0.2200^{c}
3	200	32.82 ± 0.1350^{c}	84.61 ± 0.0450^{c}	70.38 ± 0.1350^{c}	29.51 ± 0.1000^{c}	16.55 ± 0.3600^{c}
4	400	34.39 ± 0.0450^{c}	86.44 ± 0.1800^{c}	77.10 ± 0.1000^{c}	40.23 ± 0.1200^{c}	17.61 ± 0.04500^{c}
5	800	37.66 ± 0.1800^{c}	86.78 ± 0.2250^{c}	83.50 ± 0.0450^{c}	57.25 ± 0.1500^{c}	23.44 ± 0.0900^{c}

*****Data are expressed as mean ± standard deviation (N=6), mean in the same column with different subscripts are significantly different by t-test for unpaired comparison at a-p < 0.05, b-p < 0.01, c-p < 0.001, ns= non-significant, p>0.05.

Table No: 5: Tabular representation of IC50 value of drug and standard.

*Data are expressed as mean ± standard deviation (N=6), mean in the same column with different subscripts are significantly different

NAME	DPPH scavenging	Nitric oxide scavenging	FRAP	Inhibition of LPO	Phosphom-olybdenum
Ascorbic acid	117.4±0.02	2650±49.5^{c}	271.2±0.06	141.8±0.09	174.3±0.04
Fluoxetine	9724 ± 38.5^{C}	2237 ± 36.5^{a}	80.67 ± 0.67^{c}	151 ± 0.05^{b}	2802 ± 2^{c}
Duloxetine	4516 ± 5.5^{C}	2661 ± 5^{a}	80.26 ± 0.255^{c}	1665 ± 4.5^{c}	239.6 ± 0.45^{c}
Modafinil	2716 ± 45039	227 ± 3^{c}	6828 ± 4.5^{c}	1777 ± 9^{c}	5650 ± 13.5^{c}
Vilazodone	4223 ± 3^{C}	11.97 ± 0.03^{c}	109 ± 0.95^{c}	640.9 ± 0.9^{c}	3748 ± 5.5^{c}

by t-test for unpaired comparison at a-p < 0.05, b-p < 0.01, c-p < 0.001, ns= non-significant, p>0.05

DISCUSSION

2, 2-diphenyl-1-picrylhydrazine (DPPH), is composed of stable free- radical molecules. The nitrogen at position 1 of DPPH molecule contains a free electron. The antioxidants causes reduction of hydroxyl group to hydrazine resulting in the change of colour from deep violet to light yellowabsorbance of which is measured at 517nm. All drugs contains minimum of one oxygen and one nitrogen group that possess loan pair of electron in their chemical structure. The electron rich atoms (oxygen, nitrogen, sulphur) in modafinil structure should be responsible for their antioxidant potency (figure-3). [21]

Nitric oxide synthase (NOS), an enzyme forms NO and L-citrulline with O_2 and L-arginine. NO and O^-_2 produced, reacts chemically very rapidly to form peroxynitrite ($OONO^-$). This HOONO may undergo homolytic and heterolytic cleavage to form hydroxyl free radical (^-OH), Nitrogen dioxide free radical ($^{\cdot}NO_2$) and nitronium cation (NO^+_2) hydroxide anion (OH^-).These cleavage product ^-OH, NO^+ radical and NO^+_2 are most reactive damaging and cytotoxic in biological system. [22] NO also results in disturbed metabolic pathways and membrane function. It decreases protein synthesis as well as cytochrome P_{450} activation. Besides having cytotoxic effects it is one of the most important neurotransmitter, neuroprotectiveand also act as signaling molecule.It synthesizes DNA repair enzyme, decreases malarial as well as tumour cell growth. The most rapid scavenger of NO is superoxide. The chemical structure of modafinil and vilazodone have two oxygen atom that must be a contributory factor of their highest potency as antioxidant (as shown in figure-6). [23]

FRAP method determines the antioxidant potential based on the extent of chemical reduction of Fe^{3+} toFe^{2+}. Electronegative elements implies, tendency to gain electrons. It is the measure of an atom's ability to attract electron to itselfin a covalent bond, Fluorine is the most electronegative element and oxygen atom is the second most electronegative in nature. In fluoxetine structure three fluorine atom and induloxetine one oxygen atom along with a nitrogen atom is present making it more electron rich compound. More the number of available electron lone pair more is the reducing power. Hence fluoxetine and duloxetine shows best reducing power (figure- 9). [24]

In LPO method ferrous sulphate induces lipid peroxidation which means oxidative degradation of lipid. Lipid peroxidation inhibition assay of drugs inhibits the $FeSO_4$ induced lipid peroxidation in egg yolk, which is the net result of iron mediated hydroxyl radical. This can be achieved by scavenging the hydroxyl radical or by chelating the iron ions. During this free radicals generated steal electrons from the lipid in the cell membrane resulting in the cell damage. The two LPO products formed is malondialdehyde (MDA) and 4-hydroxynonenal (HNE). MDA is highly reactive carbonyl compound that for adduct with DNA base pair which are both carcinogenic as well as mutagenic. $Drug_{(s)}$ that can successfully prevent lipid peroxidation is expected to have good potential like fluoxetine. Duloxetine also shows better result (figure-12).

In phosphomolybdenum assay the total antioxidant assay is based on the reduction of Phosphate-molybdate (VI) Phosphate-molybdate (V). The change in colour on reduction from yellow to green colour is measured at695nm that determines the reducing power of each drug. Duloxetine has the maximum reducing power that can be evaluated by comparing the experimental data (shown in figure-15). [25]

CONCLUSION

Through this project it was concluded that besides having their established antidepressant property modafinil has best DPPH radical scavenging property, NO radical is scavenged by vilazodone, fluoxetine and duloxetine has almost similar FRAP radical scavenging property, fluoxetine has better potential to inhibit lipid peroxidation, and duloxetine drug is predicted to have the best phosphomolybdenum radical scavenging capacity among all the above mentioned drugs. Further confirmation is required by performing preclinical trials on these drugs *in vivo.*

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