

Effect of Ascorbic Acid on Mental Depression Drug Therapy: Clinical Study

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Abstract

Introduction: Depression disorders are very common in clinical practice. Ascorbic acid is a cofactor in synthesis of Noradrenalin from Dopamine and serotonin from tryptophan. The objective of this work was to study the effect of ascorbic acid on the action of antidepressant drugs in a clinical setting. Clinical prospective double blind study was conducted to investigate the effect of ascorbic acid on mental depression therapy and the possible therapeutic interaction with the pharmacotherapy of the disease.

Methods: Patients (outpatients) were diagnosed for mental depression according to DSM-IV, with a base line Hamilton rating scale for depression. Patients were made aware of the objectives and the methods of the study, bearing in mind the interference of their knowing with experimental results. Patients were divided into two groups; one group received a tablet of ascorbic acid with the antidepressant (Group A; n=13 at the end of the study) and the other a tablet of placebo with the antidepressant (Group B; n=9 at the end of the study), for eight weeks. Laboratory investigation was conducted for all patients, at the beginning and at the end of the study, and included complete blood analysis, lipid profile, liver function test, renal function tests, serum electrolytes and complete urine analysis. The ascorbic acid levels in plasma were measured by HPLC.

Results: Ascorbic acid with antidepressants significantly decreased the total Hamilton depression rating scale. The main overall finding from this study is that ascorbic acid was therapeutically beneficial with antidepressant in the treatment of depression, and predicts a good response in treated patients using combined ascorbic acid with antidepressants.

Conclusion: The combination of ascorbic acid with antidepressants therapy is supported; a large scale trial with placebo control is warranted; also experimental work is needed for the effect of ascorbic acid on individual antidepressant drugs.

Introduction: Depression is classified according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edn. (DSM-IV) into major depression and milder depressions. Major depression is a condition with sufficient symptoms to meet a syndrome diagnosis; the criteria for which include at least 2 weeks of five or more symptoms, one of which must be depressed mood, loss of interest or pleasure. The number of symptoms, severity of individual symptoms and resulting disability are important in deciding the severity of major depression. Milder depression is associated with significant anxiety symptoms (i.e. not meeting full syndrome diagnosis for major depression); patients with psychological responses to life stresses will often come into this category. Dysthymia is a chronic depressive state (defined as greater than 2 years duration) which does not meet full criteria for major depression [1].

Biogenic amine hypothesis proposed that the main symptoms of depression are due to a functional deficiency of the brain monoaminergic transmitters Norepinephrine (NE), serotonin (5-HT), and/or Dopamine (DA). In contrast the mania is caused by functional excess of monoamines at synapses in the brain [2]. All antidepressant drugs in clinical use increase acutely the availability of these monoamines at the synapse either by inhibiting their neuronal reuptake, inhibiting their intraneuronal metabolism, increasing their release by blocking the α_2 autoreceptors and heteroreceptors on the monoaminergic neuron, or by inhibiting the activity of monoamine oxidase enzyme which is important in the degradation of catecholamines and serotonin - since this enzyme is present in mitochondria found in nerve endings [3,4].

The acute increase in the amount of the monoamines at the synapse has been found to induce long term adaptive changes in the monoamine systems leading to the desensitization of the inhibitory autoreceptors

and heteroreceptors including the presynaptic α_2 and 5-HT_{1B} receptors and the somatodendritic 5-HT_{1A} receptors. The desensitization of these inhibitory receptors coincides with the appearance of the therapeutic response. Furthermore, the blocking of somatodendritic 5-HT_{1A} or nerve terminal α_2 receptors proved to increase the response rate in the treatment of major and treatment-resistant depression, providing further support to the assumption that the antidepressant effect results from the long-term adaptive changes in the monoamine auto- and heteroregulatory receptors [3].

Risk factors related to sociodemographic variables as male sex (particularly if single, unemployed, insecure employment, retired), substance abuse, impulsivity, feelings of helplessness and/or hopelessness, presence of anxiety, agitation or panic attacks, previous treatment or hospitalization for psychiatric illness, family history of suicide, previous suicide plans or attempts [5]. Other risk factors could be a low Social status, low educational status, low social support, weak economic status, elderly, farmer, female doctor, student, sailor, prisoners,

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immigrants, refugees, religious sect, region (uneven distribution locally by urban-rural, residential, or subculture area, spring and autumn, weekend, evening anniversary), and adverse life events such as losses and separations, criminal changes [6].

The management goals can be divided into 3 phases: The acute phase of treatment begins when the patient first presents with an episode of depression, and includes the 6 to 12 weeks required to achieve remission [7]. The initial goal of this treatment phase is to reduce existing symptoms of depression [8]. The continuation phase is the second phase of short-term antidepressants [9]. The continuation phase last from 6 to 12 months [7]. The purpose of continuation treatment is to prevent relapse; at which symptoms return [8]. The relapse occurs following premature withdrawal of medication after acute stabilization has been achieved. The maintenance phase is a long-term aspect of antidepressant treatment and can extend for years [9]. The objective of maintenance therapy is to prevent the occurrence of a new episode of depression after full recovery from previous episodes [7-9].

Antidepressants are classified into the following groups:

- Tricyclic and Tetracyclic Antidepressants (TCAs) as imipramine: its main effect is through blocking the uptake of amines by nerve terminals; the mechanism of action of TCAs includes a serotonin and norepinephrine reuptake inhibitor [10-12].
- Monoamine Oxidase Inhibitors (MAOI) as phenelzine: it increases the concentrations of NE, 5-HT and DA within the neuronal synapse through inhibition of the monoamine oxidase enzyme system. The most common adverse effect of MAOI is postural hypotension; anticholinergic side effects are common but less severe than with the TCA; sexual dysfunction is common [13].
- Selective Serotonin Reuptake Inhibitors (SSRI) as fluoxetine: it increase the concentrations of 5-HT at the synaptic cleft, enhancing serotonergic transmission. The most common adverse effect associated with SSRIs is nausea, vomiting, diarrhea, and sexual dysfunction. Gastrointestinal effects are more commonly associated with sertraline than other SSRIs. Selective serotonin reuptake inhibitors have also been associated with increased anxiety, agitation, and insomnia in a small percentage of patients. Paroxetine may be associated with daytime somnolence in approximately 20% of the patients, while fluoxetine is more likely to cause agitation and insomnia. Other side effects that are common with paroxetine include headache, dry mouth, insomnia, asthenia, sweating, constipation and tremor. Fluoxetine therapy may also cause headache, sedation, dry mouth, tremor, dizziness, fatigue, vision disturbances, and anorexia. Common side effects associated with sertraline therapy include headache, sedation, insomnia, tremor, dizziness, and fatigue [14].
- Serotonin, Norepinephrine and Dopamine Reuptake Inhibition (SNRI) as venlafaxine, at low doses, act as SSRI, at medium to high doses additional NE reuptake inhibition occurs, and at high to very high doses DA reuptake inhibition also occurs [14]. Venlafaxine has been associated with impotence, abnormal ejaculation and orgasm, especially at higher doses; it is reported to have an incidence of sexual side effects at least as high as that seen with paroxetine and sertraline [15].
- Serotonin-2 Receptor Antagonism with Serotonin Reuptake

Blockade as nefazodone and trazodone. The only difference between SSRIs and nefazodone or trazodone that 5-HT₂ receptors are blocked by nefazodone and trazodone, whereas stimulated by the SSRIs. Nefazodone and trazodone don't cause some of the side effects that SSRIs may cause such as the short-term increase in anxiety or insomnia, akathisia, and sexual dysfunction [14].

- Norepinephrine and Dopamine Reuptake Inhibitors as bupropion, acts selectively on noradrenergic and dopaminergic systems. The symptoms of dopamine deficiency could include psychomotor retardation, anhedonia, hypersomnia, cognitive slowing, inattention, and pseudodementia; such symptoms may be preferably treated by bupropion. The possible adverse effects are agitation, insomnia, nausea and seizure [13,14].
- Alpha-2 Antagonism plus Serotonin-2 and Serotonin-3 Antagonism as mirtazapine, which is an antagonist of α_2 -adrenergic autoreceptors and heteroreceptors on both NE and 5-HT presynaptic axons, and an antagonist of postsynaptic 5-HT₂ and 5-HT₃ receptors. The net effect is increased noradrenergic activity, also at 5-HT_{1A} receptors. Mirtazapine has the side effects of weight gain and sedation due to its strong antihistamine properties [14].

Ascorbic acid is one of the important soluble vitamins; humans cannot synthesize ascorbic acid due to the absence of the enzyme L-gulonolactone oxidase, while most plants and animals can synthesize ascorbic acid. Ascorbic acid has many physiological functions and antioxidant effect; in addition ascorbic acid can regenerate other antioxidants such as α -tocopheroxyl, urate and β -carotene radical cation from their radical species.

Recent evidence suggests that oxidative stress processes might play a relevant role in the pathogenic mechanism(s) underlying many major psychiatric disorders, including depression [16-22]. Reactive oxygen has been shown to modulate levels and activity of NE, 5HT, DA and glutamate, the principal neurotransmitters involved in the neurobiology of depression [22]. Moderately low levels of ascorbic acid have been linked to depression [23]. New potential targets for the development of therapeutic interventions are based on antioxidant compounds. Ascorbic acid is an antioxidant and a cofactor in the synthesis of neurotransmitters [24,25].

Aim of the work was to investigate the efficacy of ascorbic acid with antidepressants in newly diagnosed mental depressive patients.

Materials and Methods

Eight-week double blind clinical study was applied. The investigation was conducted in Ashifa Medical Center, Tripoli-Libya between February 2005 and August 2006. The study involved 99 outpatients and ended with twenty two patients. Participants were classified as drop out if they, for any reason did not finish the eight-week study protocol. Patients were made aware of the objectives and the methods of the study, bearing in mind the interference of their knowing with experimental results. Information about demographic characteristics, life style and diagnoses of the patients was recorded. Subjects came from all areas of Libya. The socio-economic status is determined by educational qualification, occupational status and residential areas of patients. The diagnoses of the patients are based on DSM-IV.

Ascorbic acid (500 mg) and placebo tablets produced by Al-Maya Pharmaceutical Factory, Al-Maya, Libya. The preparations were

administrated in orange tablets, indistinguishable in color, size and form. Both preparations were packaged in strips of 10 tablets for each.

A standard questionnaire is used to evaluate the severity of mental depression. The basic assessment of depression is done by using Hamilton Rating Scale for Depression (HAM-D 17-item) [26].

Study design

Patients underwent a standard clinical assessment comprising a psychiatric evaluation, a structured diagnostic interview and a medical history. In this double-blind study Patients were divided into two groups; one group received a tablet of ascorbic acid with the antidepressant (Group A; n=13 at the end of the study) and the other a tablet of placebo with the antidepressants (Group B; n=9 at the end of the study), for eight weeks. The codes of tablets were decoded at the end of the study after all patients completed the study. The psychiatrist assessed diagnostic status according to DSM-IV diagnostic criteria and prescribed appropriate antidepressant drugs to patients. Patients were assessed at baseline before medication started and after eight-weeks. The principal measure of the outcome was the Hamilton Rating Scale for Depression (HAM-D 17-item). The mean decrease in HAM-D score from baseline was used as the main outcome measure of response to treatment. Laboratory investigation was conducted for all patients, at the beginning and at the end of the study, and included complete blood analysis, lipid profile, liver function test, renal function tests, serum electrolytes and complete urine analysis. The ascorbic acid levels in plasma were measured by HPLC.

Clinical laboratory investigation

Laboratory investigation was performed, in Reference Medical Laboratory in Tripoli, Libya, as complete blood analysis, lipid profile include cholesterol, triglyceride and Low Density Lipoprotein (LDL), while Very Low Density Lipoprotein (VLDL) and High Density Lipoprotein (HDL) was obtained by calculations: $VLDL = \text{triglyceride} / 5$; $LDL = \text{total cholesterol} - (VLDL + HDL)$ [13]. Liver function tests include alkaline phosphates, SGPT, SGOT and T. bilirubin; renal function tests include serum urea, serum creatinine and serum electrolytes for sodium, potassium and calcium; also complete urine analysis was carried out. The ascorbic acid levels in plasma were measured by HPLC, performed in the Research Unite of Pharmacology and Clinical Pharmacy Department, Faculty of Pharmacy-University of Tripoli.

Blood sampling

Venous blood samples (10ml) were collected from patients (after overnight fasting) through hypodermic needle size 21G. Each sample was split into a part in a plain tube for biochemical measurement, a part in anticoagulants tubes, a part in EDTA tube for Complete Blood Count (CBC, Blood Count) and ESR, and a part in lithium heparin tube for Ascorbic acid evaluation [27,28].

Urine sample

Urine was collected into sterile containers. Urine samples were transported to the lab as soon as possible for routine and microscopic examination.

Plasma ascorbic acid evaluation using HPLC

The blood was stirred and then centrifuged at 15.000 rpm for five minutes; plasma was separated into micro-tubes for ascorbic acid evaluation by HPLC and frozen until assayed. Plasma samples for ascorbic acid assay were prepared by adding 0.1ml of plasma to a tube

containing 0.2ml of internal standard phenol solution (0.6 µg/ml in 0.1M perchloric acid) and 0.1ml perchloric acid. The standard solutions were prepared in ascending concentrations of ascorbic acid (100 µg/ml, 300 µg/ml and 500 µg/ml) in 0.1M perchloric acid. The mixtures of samples or standards were mixed using a vortex for 30 seconds then centrifuged using a micro-centrifuge for five minutes. The volume of 5 µl of the supernatant was injected into HPLC. The mobile phase was composed of perchloric acid: acetonitrile (89:11) mixture. The flow rate was 1ml/minute. Spectrophotometric wave length was 270 nm for ascorbic acid [29]. Retention time of ascorbic acid and internal standard were found to be in the range of 1:30 and 9:30 min respectively. The peak ratios of the samples were calculated with reference to the internal standard. Ascorbic acid levels were expressed as µg/ml.

Statistical analysis

Descriptive statistical analyses were performed using SPSS Version 10 (software package). To verify whether data were non-parametric, Kolmogrove-Simirnove maximum deviation test for goodness of fit was used. If the parameters were normally distributed, treatments were compared by one-way ANOVA; Post-Hoc test (LSD and Duncan test) was applied. If the parameters were not normally distributed, treatments were compared by the Mann-Whitney U test for un-matched sample. The differences were considered significant at the p value ≤ 0.05. The values are expressed as the mean ± standard error. Linear regression was applied for the standard solutions peak ratio, and from which the concentration of the samples is calculated using Microsoft Excel (software).

Performa of prospective study

Effect of ascorbic acid on mental depression drug therapy

I - Patient Specific Information

Name.....Age.....

Sex.....Address.....

Education.....

Professional work.....

Marital case:singlemarriedwidowdivorce

II - Personal Habit

Smoke.....

Alcohol.....

Diet.....

Exercise.....

Café addiction.....

III - History of Disease and Drug Use

Chronic disease.....

Duration of disease.....

Any present complain.....

History of menstrual cycle.....

Pregnancy and lactation.....

Obstetric.....

Drug history.....

Drug continued intake.....

Drug stop.....

IV - Physical Examination

Respiration.....

Pulse.....

Body temperature.....

Blood pressure.....

Body weight.....

Abnormal positive signs and symptoms.....

V - Investigation

-Ascorbic acid level in plasma

-Serum electrolyte

-Complete blood picture

-Kidney function test

-Complete urine analysis (pH)

-Liver function test

-Lipid profile

-Cholesterol -Triglyceride

-LDL

-HDL

-VLDL

VI-Diagnosis

VII - Present Treatment

Drug.....
 Dose.....
 Frequency of Administration.....

Hamilton depression rating scale Patient

Patient

SYMPTOMS	1 st review	2 nd review
	Pre-treatment	8 weeks later
	Date:	Date:
1. Depressed Mood		
2. Feelings of guilt		
3. Suicide		
4. Insomnia-early		
5. Insomnia-middle		
6. Insomnia-late		
7. Work & Activities		
8. Retardation		
9. Agitation		
10. Anxiety-psychic		
11. Anxiety-somatic		
12. Somatic symptoms-GI		
13. Somatic symptoms		
14. Genital symptoms		
15. Hypochondrias		
16. Weight loss either A or B		
17. Insight		
TOTAL SCORE:		

Scoring the Hamilton depression rating scale

In general, the higher total scores of HDRS is the more severe depression.

HDRS Score:	Level of depression:
10 – 13	Mild
13 – 17	Mild to Moderate
> 17	Moderate to severe

<p>1. Depressed Mood Sad, hopeless, helpless, worthless 0 = Absent. 1 = These feeling states indicated only on questioning. 2 = These feeling states spontaneously reported verbally. 3 = Communicates feeling state, nonverbal, i.e. facial expression, posture, voice and crying. 4 = Patient reports virtually only these feeling states in their spontaneous verbal and nonverbal communication.</p>
<p>2. Feelings of guilt 0 = Absent. 1 = Self-reproach, feels they have let people down. 2 = Ideas of guilt or rumination over past errors or sinful deeds. 3 = Present illness is a punishment; delusion of guilt. 4 = Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations.</p>
<p>3. Suicide 0 = Absent. 1 = Feels it is not worth living. 2 = Wishes he / she were dead or nay thoughts of possible death to self. 3 = Suicide ideas or gesture. 4 = Attempts at suicide (any serious attempt rates).</p>

<p>4. Insomnia Early 0 = No difficulty falling asleep. 1 = Complains of occasional difficulty falling asleep, i.e. more than ½ hour. 2 = Complains of nightly difficulty falling asleep.</p>
<p>5. Insomnia Middle 0 = No difficulty. 1 = Patient complains of being restless and disturbed during the night. 2 = Waking during the night; any getting out of bed rates 2 (except to go to the toilet).</p>
<p>6. Insomnia Late: 0 = No difficulty. 1 = Waking in early hours of the mourning but goes back to sleep. 2 = Unable to fall asleep again if gets out of bed.</p>
<p>7. Works and Activities: 0 = No difficulty. 1= Thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies. 2 = Loss of interest in activities, hobbies or work, either directly reported by patient, or indirectly by listlessness, indecision & vacillation; feels he/she has to push self to work or activities. 3 = Decrease in actual time spent in activities or decrease in productivity for hospitalized patients; rate 3 if he/she does not spend at least 3 hours a day in activity. 4 = Stopped working because of present illness. In hospitalized patients, rate 4 if patient engages in no activities except ward chores, or if patient fails to perform chores unassisted</p>
<p>8. Retardation: 0 =Normal speech and thought. 1 =Slight retardation at interview. 2 =Interview difficult. 3 =Obvious retardation at interview. 4 = Complete stupor.</p>
<p>9. Agitation: 0 = None. 1 = Fidgetiness. 2 =Playing with hands, hair etc. 3 =Moving about, cannot sit still. 4 =Hand wringing, nail biting, hair pulling, biting of lips.</p>
<p>10. Anxiety – psychic 0 = No difficulty. 1 = Some tension and irritability. 2 = Worrying about minor matters. 3 = Apprehensive attitude apparent in patient's face or speech. 4 =Fears expressed without questioning.</p>
<p>11. Anxiety – Somatic Symptoms can be Gastrointestinal (dry mouth, wind, indigestion, diarrhea, cramps, belching); Cardiovascular (palpitations, headaches); Respiratory (hyper-ventilation, sighing); other symptoms include: urinary frequency, sweating). 0 =Absent. 1 = Mild. 2 =Moderate. 3 = Severe. 4 = Incapacitating.</p>
<p>12. Somatic symptoms – Gastrointestinal 0 = None. 1 = Loss of appetite but continuing to eat; heavy feelings in abdomen. 2 =Difficulty eating; request or requires laxatives or medication for bowels or for GI symptoms</p>
<p>13. Somatic symptoms – General 0 = None. 1 = Heaviness in limbs, back or head; backaches, headaches, muscle aches; loss of energy and fatigability. 2 = Any clear-cut symptom rates.</p>
<p>14 = Genital symptoms Such as loss of libido, menstrual disturbances 0 = Absent. 1 = Mild. 2 = Severe.</p>
<p>15. Hypochondrias 0 = Not present. 1 = Self-absorption (bodily). 3 = Preoccupation with health. 4 = Frequent complaints, requests for help etc. 5 = Hypochondriacally delusions.</p>

16. Loss of weight check A or B

A: Rating by history.

0 = No weight loss.

1 = Probable weight loss associated with present illness.

2 = Definite weight loss (according to patient).

B: Rating by measurement.

0 = Less than ½ kg weight loss.

1 = Greater than ½ kg weight loss.

2 = Greater than 1 kg weight loss.

17. Insight

0 = Acknowledges being depressed and ill.

1 = Acknowledges illness but attributes to bad food, climate, overwork, virus, need for a rest.

2 = Denies being ill.

Results

Patients

The final sample consisted of 8 men (4 in group A, 4 in group B) and 14 women (9 in group A, 5 in group B) with mean age 33.4 years, and a range from 19 to 59 years. 54.5% of patients lived in Tripoli city and 45.4% lived out of Tripoli city. 12 patients were singles (6 of each sex), married females were 7, married males were 2, and 1 divorced female. Of the 22 patients, 7 completed primary education, 10 completed lower secondary educations, 2 completed higher secondary, and 3 completed higher education. There were 8 professionals among the sample, 2 students, 4 unemployed, and 8 house wives.

None of the patients drank alcoholic beverages nor exercised, while smoking was reported by 4 males (3 in group A; 1 in group B). Coffee drinker was reported in 5 females and 2 males in group A, while it was reported in 2 females and none of males in group B. Regarding the diet habit of patient, occasionally 18 patients depends on carbohydrates, while 5 patients were depends on carbohydrates, fresh vegetables and fruits in their diet.

The patients diagnosed as major depression disorder were 8; those as depression with panic attack were 8; those as depression with obsessive compulsive disorder 2; those as psychotic depression were 3; and 1 diagnosed as depression with agoraphobia.

Diagnosis of the patients

In group A, 8 (61.53%) were treated with paroxetine 20mg, 2 (15.38%) were treated with fluoxetine 20mg, 1 (7.69%) was treated with combination of olanzapine 10mg and clomipramine 75mg, 1 (7.69%) was treated with clomipramine 75mg, and 1 (7.69%) was treated with fluvoxamine 20mg. In group B, 5 (55.55%) were treated with paroxetine 20mg, 1 (11.11%) was treated with combination of olanzapine 10mg and fluoxetine 20mg, 1 (11.11%) was treated with combination of quatiptine 200mg and clomipramine 75mg, and 2 (22.22%) were treated with clomipramine 75mg. Nitrazepam 5mg was prescribed in two cases of group A and one of group B.

Hamilton Depression Rating Scale (HDRS-17)

As shown in group A, the total scores of HDRS-17 were significantly decreased from (21 ± 1.25) before treatment to (5.38 ± 1.01) after treatment ($P=0.000$); in this group, the symptoms of depression were significantly decreased after the combined treatment of ascorbic acid and antidepressant drug compared to the same group before treatment (Table 1). The symptoms of depression that were significantly decreased were depressed mood ($P=0.000$), feeling of guilt ($P=0.000$), suicide ($P=0.017$), early insomnia ($P=0.006$), middle insomnia ($P=0.004$), late insomnia ($P=0.004$), work and activities ($P=0.001$), agitation ($P=0.001$), anxiety psychic ($P=0.000$), anxiety somatic ($P=0.000$),

somatic symptom-GI ($P=0.002$), somatic symptom ($P=0.002$), genital symptoms ($P=0.022$), hypochondrias ($P=0.001$); while there is no significant differences in score of retardation, weight and insight compared to pretreatment.

In group B, there is significant decrease in the total score HDRS-17 from (22.33 ± 1.9) before treatment to (11.67 ± 2.42) after treatment ($P=0.001$); in this group there is significant decrease in the symptoms of depression after treatment with combined placebo and antidepressant drugs compared to the same group before treatment. The symptoms of depression that were significantly decreased are depression mood ($P=0.031$), feeling of guilt ($P=0.024$), early insomnia ($P=0.039$), middle insomnia ($P=0.009$), late insomnia ($P=0.047$), weight loss ($P=0.008$); while there is no significant differences in the score of work and activities, retardation, agitation, suicide, anxiety psychic, anxiety somatic, somatic symptom-GI, somatic symptom, genital symptoms, hypochondrias and insight.

When comparing the two groups (A and B) after treatment, it was found that there are significant decreases in the scores of agitation ($P=0.01$), anxiety psychic ($P=0.02$), anxiety somatic ($P=0.021$), hypochondrias ($P=0.003$), and total scores ($P=0.014$). On the other hand, when comparing the two groups before treatment, no significant difference was observed.

Physical examination

There was no significant changes in physical examination parameters such as respiratory rate, pulse, body temperature, systolic blood pressure, and diastolic blood pressure between groups A and B before and after treatments ($P>0.05$), while body weight showed a significant decrease among group B after treatment in comparison to before treatment.

Laboratory investigation

The hematology parameters RBC, HGB, HCT, MCV, MCH, MCHC, neutrophils, lymphocyte and PLT were not changed significantly ($P>0.5$) before and after treatment in both groups A and B. While WBC and ESR was significantly increased in group B after treatment ($P=0.048$ and 0.046 respectively) compared to group A after treatment, although all the parameters were within the normal ranges.

Kidney function parameters (urea, s. creatinine, potassium and sodium) were not significantly changed before and after treatment in both groups A and B ($P>0.05$). While serum calcium was significantly increased in group B after treatment ($P=0.016$) compared to the same group pre-treatment and group A after treatment, although all the levels were within the normal range.

Cholesterol, triglyceride, VLDL, HDL and LDL were not significantly changed before and after treatment in both groups A and B ($P>0.05$).

Liver function parameters (alkaline phosphates, S.G.P.T, S.G.O.T and T. bilirubin) were not significantly changed before and after treatment in both group A and B at $P > 0.05$.

Protein, glucose, acetone, urobilinogen, bilirubin, nitrate were absent in all samples before and after treatment in both groups A and B. Other parameters (pH, epithelial cell, erythrocyte, pus cell) were not significantly changed before and after treatment in both groups A and B ($P>0.05$).

Symptoms	Treatment without ascorbic acid		Treatment with ascorbic acid	
1. Depressed Mood	2 ± 0.16	0.54 ± 0.24*	1.89 ± 0.20	1 ± 0.29*
2. Feeling of guilt	2.08 ± 0.24	0.38 ± 0.18*	2.22 ± 0.22	1 ± 0.37*
3. Suicide	1.31 ± 0.35	0.31 ± 0.17*	1.56 ± 0.38	0.67 ± 0.33
4. Insomnia-early	1.23 ± 0.17	0.46 ± 0.18*	1.33 ± 0.24	0.67 ± 0.24*
5. Insomnia middle	1 ± 0.23	0.15 ± 0.1*	0.89 ± 0.26	0.11 ± 0.11*
6. Insomnia-late	0.77 ± 0.20	0 ± 0*	0.89 ± 0.26	0.22 ± 0.15*
7. Work &Activities	2.46 ± 0.31	0.77 ± 0.32*	1.89 ± 0.35	1 ± 0.33
8.Retardation	0 ± 0	0 ± 0	0.22 ± 0.22	0.11 ± 0.11
9. Agitation	1.08 ± 0.21	0.15 ± 0.1*	1.44 ± 0.34	1 ± 0.29 ^a
10. Anxiety-psychic	2.31 ± 0.17	0.69 ± 0.17*	2 ± 0.29	1.56 ± 0.29 ^a
11. Anxiety-somatic	1.77 ± 0.12	0.46 ± 0.14*	1.67 ± 0.24	1.22 ± 0.22 ^a
12. Somatic-symptom GI	0.92 ± 0.18	0.15 ± 0.10*	0.89 ± 0.26	0.56 ± 0.24
13. Somatic-symptom	1.15 ± 0.19	0.46 ± 0.14*	1.33 ± 0.29	0.78 ± 0.28
14.Genital-symptoms	1 ± 0.23	0.31 ± 0.17*	0.89 ± 0.26	0.44 ± 0.24
15. Hypochondrias	1.23 ± 0.38	0 ± 0*	1.67 ± 0.37	0.78 ± 0.28 ^a
16. Weight loss (B)	0.62 ± 0.24	0.62 ± .018	1.33 ± 0.29	0.33 ± 0.17*
17. Insight	0.08 ± 0.077	0 ± 0	0.22 ± 0.15	0.22 ± 0.15
Total score	21.08 ± 1.26	5.46 ± 1.06*	22.33 ± 1.9	11.56 ± 2.4 ^a

*, significantly different from pretreatment at $P \leq 0.05$; a, significantly different from group A at $P \leq 0.05$; group A = ascorbic acid & antidepressant; group B = Placebo & antidepressant

Table 1: Hamilton depressions rating scale.

Plasma ascorbic acid levels

The standard solution peak ratio for ascorbic acid curve were linear ($R^2=0.99$). Plasma ascorbic acid levels were significantly increased in group A after treatment compared to the same group before treatment ($P=0.029$); while there is no significant changes in the plasma ascorbic acid level in group B before and after treatment. Plasma ascorbic acid levels were significantly increased in patients of group A after treatment compared to patients in group B after treatments. All the levels of ascorbic acid in different treatment were within the normal range (Table 2).

Discussion

Patients

In a double-blind trial, neither patients nor treating physicians know which patient is given drug and which is given placebo [30]. The study was carried out by interview that is kept blind; a double blind trial was applied.

Although the number of patients is small, it may show an indication about lifestyle and the management of mental depression. The present study showed that the disease is more prevalence in women than men (63.63% vs. 36.36%). These observations are consistent with the findings of other studies in literature which have shown that women experience depression about twice as often as men [31]. While in the other study the female: male ratio can be as high as 5:2 [32]. Many hormonal factors may contribute to the increased rate of depression in women particularly factors as menstrual cycle changes, pregnancy, postpartum period, pre-menopause, and menopause. Many women also exposed to additional stresses such as responsibilities both at work and home [31].

Noticeable, the females were more café drinker than males and this is another reason for prevalence of depression is more in female than male; the consumption of one or two cups of café, acts as a non-specific antagonist of both A1 and A2 adenosine receptors. Adenosine, by acting at the level of presynaptic A1 receptors, inhibits the release of numerous neurotransmitters such as glutamate, GABA, acetylcholine and monoamines and is more efficient at the level of excitatory than inhibitory neurotransmission. Thus, caffeine by antagonizing the effects

Parameters & (Normal value)	Group A (n=13)		Group B (n=9)	
	Pre-treatment	Post- treatment (AA & AD)	Pre-treatment	Post-treatment (Placebo & AD)
Plasma ascorbic acid (5µg/ml-15µg/ml)	4.45 ± 0.27	7.49 ± 1.5*	5.62 ± 0.7	5.14 ± 0.49 ^a

The values are the means ± S.E; Ascorbic acid levels were expressed as µg/ml; *, significantly different from pre-treatment group A or B at $P \leq 0.05$; ^a, significantly different from group A after treatment at $P \leq 0.05$; AA, ascorbic acid; AD, antidepressant drugs

Table 2: Effect of antidepressant drugs on plasma ascorbic acid level in presence of ascorbic acid.

of endogenous adenosine increases the firing rate of central neurons. Adenosine is an extremely important anti-anxiety and antidepressant molecule produced by the brain and caffeine specifically antagonizes these effects [33].

Regarding the patients' marital status, nearly higher percentage of female patients (31.8% vs. 9.09%) was married compared to males; while equal percentages (27.2% vs. 27.2%) of males and females patients were single. These findings indicate that married women are more suffering from depression as compared to single women. In educational status, the percentage of patients with basic primary and intermediate school (31.8%, 45.5%) respectively was higher than the percentage of patient with higher level school and graduate qualification (9.09%, 13.6%) respectively. The percentage of depressed patients were living in Tripoli city nearly 54.5% while 45.4% living out of Tripoli city. Regarding the occupational status of patients, the house wives and patient with professional work were more percentage of depression suffering than jobless and students.

In this study, the patients (male and female) were not alcohol drinker and even with no exercise program; the females were nonsmoker while 50% of males were smoker. The café drinker was 50% in females verses 25% in males; this is a factor for prevalence of depression in females more than males in this study. The mental depression disease may be present alone and represented about 36.36%; it might be associated with other disease such as anxiety and represented nearly 49.99% (total of depression with panic attack, depression with obsessive compulsive

disorder, depression with agoraphobia), and psychotic depression 13.63%.

The Hamilton depression scale has been the standard for the assessment of depression for more than 40 years [34]. A large number of studies have shown that it is a valid and reliable measure of depression. Also it is sensitive to changes due to drug effects in a general practice setting [35,36]. The structured 17-item interview version of Hamilton Depression Rating Scale (HDRS) is being used in this study.

As mention before in the results (Table 1), after treatment in group A, there is a significant decrease in the suicide, agitation, anxiety (psychotic and somatic), hypochondrias, and total scores, compared to after treatment group B. Brody (2002) found that ascorbic acid but not placebo group produces a decrease in Beck Depression scores and this agrees with the results of this study[2]. These symptoms were not improved significantly by administration of antidepressants and placebo, they may need more time than eight weeks of treatment to show significant improvement.

In older people, low levels of body ascorbic acid were correlated with both depression and higher mortality rates [23]. A history of attempted suicide is associated with low levels of antioxidant vitamins [37]. Suicide attempts occur due to certain biological variables, which were related to changes in serotonin neurons (low activity of serotonin); low concentration of the serotonin metabolite 5-hydroxy indol acetic acid and high cortisol production was associated with suicide attempts [38]. Ascorbic acid modulates decreases stress reactivity may be through the decrease of cortisol synthesis [39].

Ascorbic acid caused a synergistic antidepressant-like effect with conventional antidepressants [40]. Dysfunction of serotonergic neurotransmission has been associated with several mood disorders, including depression, anxiety, panic disorder, obsessive compulsive disorder and eating disorders. Drugs which increase the levels of serotonin, such as selective serotonin uptake inhibitors, have anxiolytic effects, particularly in patients suffering from generalized anxiety and panic disorders [41]. Previous studies performed by Naidu (2003) reported that a deficiency of ascorbic acid leads to depression, because ascorbic acid is a cofactor for dopamine β -hydroxylase and formation of norepinephrine[25]. Norepinephrine specific activity was increased by ascorbic acid; ascorbic acid is necessary for the conversion of tryptophan to 5-hydroxy tryptophan "serotonin" and the formation of the neurotransmitter, epinephrine from dopamine[42]. The deficiency of ascorbic acid can cause neurotic disturbances consisting of hypochondriasis, hysteria and depression followed by decreased psychomotor performances[43].

In this study agitation, anxiety (psychotic and somatic), and hypochondrias were significantly decreased in after-treatment of group A compared to after-treatment of group B. Ascorbate is released from glutamatergic neurons, in which the high affinity glutamate transporter exchanges ascorbate for glutamate [44]. Glutamate induces excitation in mood disorders, and can reduce the GABA response by auto-desensitization [45,46]. Ascorbate may alter the redox state of NMDA glutamate receptor, thus block NMDA gated channel function, also the antidepressants may act as functional NMDA receptor antagonists; the antagonism of NMDA receptors exhibit an anxiolytic and antidepressant like activity in animals [44,47,48]. A preclinical and clinical research suggests that NMDA class of glutamate receptors may be involved in the pathophysiology of major depression disorder and the mechanism of action of antidepressants; NMDA receptor antagonists had dose-related effects that were comparable in magnitude, but more rapid, than imipramine [49].

Agitation is a pathophysiologic abnormality mediated by dysregulations of dopaminergic, serotonergic, noradrenergic, and GABAergic systems. Agents that reduce dopaminergic or noradrenergic tone or increase serotonergic or GABAergic tone will attenuate agitation, often irrespective of etiology [50]. Ascorbic acid amplifies the action of anti-dopaminergic drugs; ascorbic acid attenuates the behavioral effects of amphetamine and potentiates the behavioral response to haloperidol [51,52]. Some of these behavioral effects, however, may be dose-dependent in that treatment with relatively low doses of ascorbate has been reported to enhance dopamine-mediated behaviors. Ascorbate also may alter the redox state of the NMDA glutamate receptor thus block NMDA-gated channel function; such action decreasing the influx of calcium ions into the neuron, and hence downregulates the activation of calcium dependent enzymes, this can prevent the neuronal excitotoxic damage; glutamate mediated an inhibitory postsynaptic potential in dopamine neurons [44,53,54].

A high level of traumatic anxiety, reflected by elevated heart rate, is indicative of augmented catecholamine release. Immediate increase in glutamate efflux in prefrontal cortex and hippocampus were observed after induction of acute stress. NMDA receptors may mediate an increase in anxiety-like behavior produced by the stress of predator exposure [55]. The micro injection of glutamate into the Para ventricular nucleus produced dose-dependent increases in blood pressure, and heart rate. These responses were blocked by the NMDA receptor antagonist. In contrast, GABA is a well-known inhibitory neuro transmitter in the central nervous system, and plays an important role in central sympathetic and cardiovascular regulation. Intracerebroventricular injections of GABA agonists decrease arterial blood pressure and heart rate; ascorbic acid stimulates GABA binding [56,57]. Also ascorbic acid role might be due to block NMDA-gated channel function [44]. A study suggests that reduced DA function is related to social anxiety [58]. The effect of ascorbic acid on the dopaminergic system is markedly dose dependent; a low dose (100 mg/kg i.p.) potentiated the dopaminergic action while higher doses (400-1,600 mg/kg i.p.) blocked it[51]. Ascorbic acid may relieve anxiety and stress either through stimulated GABA binding and block NMDA-gated channel function, or may act through activation of dopaminergic system.

Ascorbic acid may modulate catecholaminergic activity and decreases stress reactions; the evidence is from a case study in which ascorbic acid was used to treat obsessive-compulsive disorder [59]. Ascorbic acid is postulated as a factor regulating the stimulation of catecholamine on $\text{Na}^+\text{-K}^+\text{-ATPase}$ and there by the turnover of neurotransmitters in the central nervous system tissues [60].

Patients with major depression are exposed to oxidative stress with decreased levels of plasma ascorbic acid [61,62]. Ascorbic acid is able to scavenge a broad spectrum of reactive oxygen, and is therefore one of the most important exogenous antioxidants in body [63]. Ascorbate catalyzes the reduction of superoxide radicals, and may provide protection against the oxidative stress [64]. Ascorbic acid acts as co-factor for dopamine- β - hydroxylase activity, which catalyzes the conversion of neurotransmitter dopamine to norepinephrine [25,65].

Deficiency of ascorbic acid can cause neurotic disturbances consisting of hypochondriasis, hysteria and depression followed by decreased psychomotor performances [43]. Administration of ascorbic acid improved the hypochondrias.

Ascorbic acid deficiency causes exhaustion, a decrease in performance during physical activities, increased muscle pain [66]. Muscle weakness is a common feature of clinical carnitine deficiency;

ascorbate is a cofactor of two-enzyme: hydroxylation in the pathway of carnitine biosynthesis and ascorbic acid is a co-factor for the conversion of lysine to carnitine [67,68]. Intake of ascorbic acid in amounts lower than the necessary detrimentally affects exercise performance. It has been reported that ascorbic acid intake must be increased with increases in physical exercises [66].

Ascorbic acid reduces the somatic-symptom (GI) significantly; its deficiency causes lack of appetite [66]. Ascorbic acid is a natural laxative and may help with constipation problem, potentiated the effect of acetylcholine[43].The contractile effect of ascorbic acid was potentiated by eserine and inhibited by atropine. These indicate to a stimulatory effect of ascorbic acid on the muscarinic receptor [69].

Ascorbic acid reduces the somatic symptoms by exerting its anti-nociceptive effects primarily as a result of its antioxidant properties. Ascorbic acid may have a role to play in migraine prophylaxis; also it has been helpful for relief of back pain [43,70]. Ascorbic acid at low systemic doses produces a rapid onset and consistent anti-nociception in mice when assessed in several models of chemical nociception; it produced significant inhibition of glutamate-induced nociception that is likely mediated by an interaction with ionotropic, but not metabotropic glutamate receptors [71].

Depression and anxiety may cause sexual dysfunction, which can be temporary or long-lasting. Antidepressants may help, although some antidepressants may cause low libido [72-74]. Ascorbic acid may help both men and women, as it increases blood flow via nitric oxide-dependent mechanisms [75,76]. One study suggests that ascorbic acid may increase libido in women [75]. Ascorbic acid "stimulate endothelial NO synthesis; boosting NO output and increasing blood flow [77,78].

Healthy young males found that Ascorbic acid increased the intercourse frequency [2]. Plasma levels of FSH, LH and testosterone significantly decreased in rats exposed to noise stress. Administration of ascorbic acid increased significantly the levels of hormones into normal range [79].

Ascorbic acid neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals and prevents sperm agglutination [80]. Ascorbic acid supplementation in infertile men might improve sperm count, sperm motility, and sperm morphology [81].

Poor progesterone production could be due to oxidative stress, free radicals, and high levels of lipoperoxide. Ascorbic acid helps to prevent oxidative stress and the production of free radicals that interfere with progesterone production [82,83]. Ascorbic acid appears to increase penile-vaginal intercourse, and also experienced a decrease in Beck Depression scores[2]. Libido dysfunction may occur due to $\alpha 1$ adrenergic receptor antagonism, $\alpha 2$ adrenergic receptor antagonism, 5-HT reuptake inhibition, 5-HT_{2A/2C} receptor antagonism [84]. Male rabbits treated with ascorbic acid, vitamin E, and their combination significantly increased libido (reaction time) and ejaculation volume [85].

Ascorbic acid protected from weight loss. Decrease brain serotonin levels at postsynaptic sites, leading to depression. A compensatory upregulation of postsynaptic 5-HT-1A receptors and hypophagic serotonin receptors may be involved in anxiety and suppression of appetite [86]. During vitamin C deficiency, weight loss was observed in mice; this returned to control levels following administration of ascorbic acid [87]. Rapid weight loss can cause an extreme hormonal imbalance that can lead to mood swings, difficulty concentrating, stress and anxiety [88].

Ascorbic acid administration did not show any significant changes

in insomnia, depressed mood, feeling of guilt compared to placebo treatment. Retardation and insight were not affected by treatment with antidepressants with or without ascorbic acid for eight weeks.

Yaniket al. (2004) reported that patients with major depression are exposed to oxidative stress. Major depression is associated with increased levels of serum superoxide dismutase and decreased levels of plasma ascorbic acid. Treatment with fluoxetine and citalopram reversed these biochemical parameters [61,62]. Ascorbic acid is able to scavenge a broad spectrum of reactive oxygen, and is therefore one of the most important exogenous antioxidants in the body [63]. Under certain conditions where there is an increase in the amounts of oxy radicals and reactive semiquinone/quinone intermediates are formed from catecholamine neurotransmitters, a number of cellular mechanisms may step in to limit the extent of oxidative neuronal damage in certain regions of the CNS. The presence of reducing compounds, such as ascorbate, and the protective enzymes, such as superoxide dismutase that catalyzes the reduction of superoxide radicals, may jointly provide protection against the oxidative stress caused by elevated levels of oxy radicals [64].

Ascorbic acid is taken up and stored by brain cells and modulates the binding characteristics and the function of neurotransmitter receptors including those for 5-HT [89]. In the brain, the nerve endings contain the highest concentrations of ascorbic acid in the human body [90]. Ascorbic acid participates in a myriad of biochemical reactions in which it maintains enzymes involved in reactions such as catecholamine synthesis, and tyrosine metabolism. Although ascorbic acid did not penetrate the blood-brain barrier, its oxidized form dehydroascorbic acid readily entered the brain by means of facilitative transport. The facilitative Glucose Transporter (GLUT1) present on endothelial cells at the blood-brain barrier is responsible for transport of both glucose and dehydroascorbic acid into the brain. Ascorbic acid concentrations in the brain exceeded those in blood by tenfold [91]. Hediger (2002) reported that dehydroascorbate may exit the neurons through facilitated transport via GLUT3 or other transporters and then enter astrocytes via GLUT1. Inside astrocytes, dehydroascorbate is reduced back to ascorbic acid, which may be released and taken up again by neurons via Sodium-Dependent Vitamin C Transporters (SVCT2) [92]. This cycle can explain the predominant localization of ascorbic acid in neurons.

Relatively high concentrations of ascorbic acid as well as early expression of SVCT2 have been detected in the fetal brain suggesting that ascorbic acid might have a protective role during brain development as well; Dopamine- β -hydroxylase is a blood marker of unipolar depression. Previous research studied the effect of imipramine on activity of this enzyme in plasma of rats exposed to chronic mild stress (anhedonia model); it was found that imipramine treatment minimized these chronic mild stress induced reductions in dopamine- β -hydroxylase activity [93]. Ascorbic acid acts as co-factor for the enzyme dopamine- β -hydroxylase activity, which catalyzes the conversion of neurotransmitter dopamine to norepinephrine [25,65].

Short-loop regulatory circuits are found within the dorsal raphe nucleus and the adjacent periaqueductal gray (PAG). These short-loop circuits involve interaction between 5-HT and local inhibitory GABAergic (γ -aminobutyric acid) and excitatory glutamatergic neurons. Local GABAergic neurons are activated by 5-HT via 5-HT_{2A/2C} receptors in a local, negative feedback loop that complements 5-HT_{1A}-mediated autoinhibition. Neurokinins such as substance P and neurokinins B, via NK₁ and NK₃ receptors, respectively, activate mostly local glutamatergic excitatory inputs to 5-HT cell; ascorbic acid causes a concentration-dependent increase in the affinity of 5-HT for

central 5-HT₃ binding sites; in hippocampus, 5-HT has been reported to increase GABAergic inhibitory postsynaptic potentials (IPSPs), through 5-HT₃ receptor mediated excitation of inhibitory interneurons [94]. The Ca²⁺ entry via presynaptic 5-HT₃Rs facilitates the release of GABA from hippocampal interneurons [95]. Ascorbic acid at a concentration nearly equal to brain extracellular one (3×10^{-4} M), had no effect on GABA binding. At higher concentrations (10^{-3} M), ascorbic acid strongly inhibited, and at lower concentrations (10^{-6} M) considerably stimulated GABA binding. At a concentration of 10^{-5} - 10^{-3} M ascorbic acid tended to decrease 3H-DL-glutamic acid binding [57].

The locus ceruleus, raphe nuclei and ventral tegmental area receive a variety of neuronal inputs, including the monoamines themselves that regulate their activity. Several neurotransmitter inputs to monoamine nuclei are particular relevance to major depression because of the accumulation of evidence that these systems are also disrupted in depression. For example, abnormalities in GABA, substance P, corticotrophin releasing factor (CRF) and glutamate neurochemistry have been implicated in depression; depression is associated with reduced GABA function, increase in glutamate level, substance P and CRF [96].

In general, the administration of ascorbic acid combined with the antidepressant drugs reduced Hamilton depression rating scale compared to the antidepressant drugs effect without ascorbic acid administration. This indicates that ascorbic acid improves the antidepressant drugs action and reduces their side effects.

Physical examination

The physical examination parameters in both groups A and B before and after-treatment are within the normal values; these indicate that ascorbic acid or antidepressants do not produce any deterioration in these parameters during the eight weeks treatment.

Hematology

The results of hematology parameters were normal in values, no significant changes before and after treatment in both groups A and B. Noticeably, WBC and ESR were significantly increased in group B after treatment compared to group A after treatment, although the parameters are within the normal range. This indicates that ascorbic acid or antidepressant does not produce any abnormality in these parameters.

Biochemical investigation

Ascorbic acid keeps the calcium levels in normal range, in group A compared to the placebo treatment (group B) that showed a significant increase in calcium levels; although the calcium levels in both groups are within the normal range. This result might be related to the effect of ascorbic acid on vitamin D; ascorbic acid deficiency in guinea pigs fed a vitamin D-replete diet caused a moderate reduction of Ca²⁺ level in serum and bone. Ascorbic acid deficiency potentiated effects of vitamin D deprivation and impaired a restorative action of vitamin D. The effects of ascorbic acid on vitamin D hormonal system function, is manifested both at the level of 1.25-(OH) 2D₃ synthesis in the kidneys and of its receptor binding in target tissues [97]. It was accompanied by a marked delay in the elevation of 25-hydroxyergocalciferol concentration in serum as well as decreased 1-OHase activity in kidneys and a lower concentration of occupied 1.25-dihydroxyvitamin D₃ receptors in the intestinal mucosa. The data demonstrate a critical role for ascorbic acid in vitamin D metabolism and binding [98].

Kidney function parameters, lipid profile, liver function parameters

were within the normal range with no significant differences between the two groups (A&B). Although Ascorbic acid helps to lower blood cholesterol and has a role in its metabolism leading to increase its elimination [43]. Also ascorbic acid is necessary for the transformation of cholesterol to bile acids as it modulates the microsomal 7 α -hydroxylation, and the rate limiting reaction of cholesterol catabolism in liver [25]. Ascorbic acid in doses of approximately 1g daily has shown to help protect the body against Low Density Lipoprotein [99]. In this study the dose of ascorbic acid may be needed to be adjusted to produce any changes in the lipid profile.

Urine analysis

Protein, glucose, acetone, urobilinogen, bilirubin, and nitrate were absent in all samples before and after treatment in both groups A and B and these comply with the normal range.

The other parameters (pH, epithelial cell, erythrocyte, and pus cell) were not significantly changed before and after treatment in both groups A and B. All the parameters were within the normal range except erythrocyte was (3.13/hpf) above the normal value (0-2/hpf) and these may resulted from contamination of the urine during collection by the blood of menstrual cycle in some females.

Plasma ascorbic acid level

The plasma ascorbic acid levels significantly increased in group A after-treatment while there is no significant change in group B. In this study the plasma ascorbic acid is within the normal range of 5 μ g/ml-15 μ g/ml. Eekelen (1936) found that the content of ascorbic acid in blood and its urinary output depended on the amount of ascorbic acid taken in and on the quantity stored in the organism. Furthermore, Khassafet al. (2003), found that there is an increase in serum ascorbic acid concentration by about 50% after ascorbic acid supplementation at a dose of 500 mg per day for 8 weeks [100,101]. The average half-life of ascorbic acid in adult human is about 10-20 days [25]. Higher plasma levels of 800 to 900 mg/ml after chronic intake of ascorbic acid have been observed in individuals. A higher supplementation with time might have produced higher plasma levels [102].

Valdés (2006) found that the concentrations of ascorbic acid in plasma and leukocytes reflect the levels of the diet and body deposits respectively [103]. The current recommendation of daily intake of ascorbic acid is 90mg/d for men and 75mg/d for women, patients with chronic diseases such as cancer or diabetes or those who smoke need higher doses in their usual diet.

Conclusion

In this clinical study, combined administration of ascorbic acid with antidepressants decreased significantly the total HDRS scores. Laboratory investigations were within normal values. The main overall finding from this study is that ascorbic acid was therapeutically beneficial with antidepressants, and it predicts a good response in treated depressed patients. Finally, this clinical study supports the role of combined ascorbic acid with antidepressants and a large scale trial with placebo control is warranted; also experimental work is needed for the effect of ascorbic acid on individual antidepressant drugs.

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