e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(2); 140-147

Original Research Article

γ-Glutamyl Transferase as a Marker of Oxidative Stress in Pre and Post-Menopausal Women

Stuti Soni[1], Gora Dadheech[2], Mamta Singh[3], R.C. Gupta[4]

[1]PG student, Department of Biochemistry, NIMS Medical College, Jaipur, Rajasthan
[2]Assistant Professor, Department of Biochemistry, Dr. S.N. Medical College, Jodhpur, Rajasthan
[3]Assistant Professor, Department of Biochemistry, S.M.S. Medical College, Jaipur, Rajasthan
[4]Professor, Department of Biochemistry, NIMS Medical College, Jaipur, Rajasthan

Received: 30-11-2022 / Revised: 30-12-2022 / Accepted: 30-01-2023
Corresponding author: Dr Gora Dadheech
Conflict of interest: Nil

Abstract

Introduction: Menopause is a physiological process in women representing complete cessation of menstruation and gradual decrease of estrogen secretion. The antioxidant substances present in the body play a role in combating the oxidative stress in menopause. Since, the enzyme γ-glutamyl transferase plays an important role in the metabolism of the endogenous antioxidant reduced glutathione (GSH), it can reflect the antioxidant ability *in vivo*.

Aim: The present study was conducted to investigate γ-glutamyl transferase as a possible marker of total antioxidant capacity (TAC) of plasma.

Materials & Method: The study included 50 female volunteers, selected from medical & paramedical staff, and healthy attendants of patients as per inclusion and exclusion criteria and were divided into two groups.

Group I: Premenopausal women (n = 25);

Group II: Postmenopausal women (n = 25). Venous blood samples were collected using aseptic techniques in plain vials for estimating estradiol hormone and Gamma GT and whole blood was collected in EDTA vials for the estimation of Ferric reducing ability of plasma (FRAP) which is a marker of antioxidant capacity. The data collected were analyzed using unpaired "t"-test for evaluating the level of oxidative stress in pre and post-menopausal women and the variations in the levels of gamma GT in the two groups. The results were expressed as Mean ± Standard Deviation (SD).

Results & Conclusion: Depressed estrogen synthesis in postmenopausal women enhanced oxidative stress and lead to deficit of total antioxidant capacity of plasma. A significant increase in serum GGT in the post-menopausal group relative to the pre-menopausal group was found which was to compensate and correct the reduced glutathione levels by increasing its biosynthesis. Thus, GGT can be used as a marker of total antioxidant capacity of plasma and oxidative stress.

Keywords: Oxidative Stress, Γ-Glutamyl Transferase, Total Antioxidant Capacity, Menopause.

Introduction

Midlife menopause, which happens to women in their late 40s or early 50s, marks the end of the fertile stage of a woman's life [1,2]. As an immune-modulating hormone, oestrogen is linked to healthy immune system function, which in postmenopausal women may be affected, as oestrogen production declines after menopause. The immune system has a variety of defences that aid in preventing the onset of certain diseases, some of which are age-related [3,4]. In addition to its primary hormonal impact, estradiol probably has two mechanisms that act as antioxidants. One of them is the outcome of the molecule's hydroxyphenolic structure. Estradiol can stop the chain reactions of lipid peroxyradicals by donating hydrogen atoms from its phenolic hydroxyl group [5]. Estradiol also inhibits oxidative modification of LDL lipoprotein which plays the main role in atherogenesis. The other mechanism of antioxidative action of estradiol is probably associated with its stimulatory effect on natural cellular antioxidant enzymes. An increase in oxidative stress and a decrease in estrogen place postmenopausal women at increased risk for several diseases [6,7].

An imbalance between the formation of free radicals and reactive metabolites, also known as oxidants, and their removal by protective systems, also known as anti-oxidative systems, is referred to as oxidative stress [8]. Normally, antioxidants neutralize reactive oxygen species (ROS) and thus help to prevent over exposure from oxidative stress [9]. Three conditions that deal with the role of oxidative stress in aging include: 1) levels of molecular oxidative damage increase during aging; 2) a relatively longer life expectancy within and among species is associated with a correspondingly lower accrual of oxidative damage; 3) a prolongation of life-span by regimens such as caloric restriction in mammals is associated with the amelioration of oxidative damage [10].

Gamma-glutamyl transferase (GGT) which is an enzyme involved in the transfer of the γ-glutamyl residue from γ-glutamyl peptides to amino acids, H_2O, and other small peptides that can be donated by glutathione. In most biological systems, glutathione, an endogenous antioxidant serves as the γ-glutamyl donor [11]. On the other hand, GGT is also involved in the synthesis of glutathione [12]. The intracellular glutathione (GSH) level depends upon the equilibrium between processes during which it is consumed and its biosynthesis is limited by cysteine availability [13]. The recovery and delivery of cysteine is the purpose of the -glutamyl cycle. Gamma glutamyl transpeptidase (GGT) activity is necessary for the availability of cysteine, a building block needed for the manufacture of cellular glutathione, the most significant cell antioxidant, hence this enzyme may be crucial to the cell's anti-oxidative defence mechanism [14]. The total antioxidant capacity or antioxidant activity of all the chemicals present in plasma can be used to measure oxidative stress [15]. The level of GGT might be regarded as an index or a marker of oxidative stress since it plays a role in the regeneration of reduced glutathione. Several markers have been explored in menopausal women, but no precise conclusions have been obtained [16].

The aim of the present study is to evaluate the oxidative stress as total antioxidant capacity of plasma in pre and post-menopausal conditions and to find the variation in level of γ-GT as a marker of oxidative stress associated with menopause in women.

Material & Methods

The present study was carried out in the Department of Biochemistry, NIMS Medical College and Hospital, Shobha Nagar, Jaipur, Rajasthan in collaboration with the Department of Obstetrics and Gynaecology. The protocol of the study was approved by

the Institutional Ethical Committee, NIMS Medical College, Shobha Nagar, Jaipur, and informed signed consent was given by each subject.

It included 50 female volunteers, selected from medical & paramedical staff, and healthy attendants of patients. The personal and clinical history of all subjects was recorded with the help of a questionnaire. Clinical examination was carried out by a competent gynaecologist. The subjects were categorized into following groups:

Group I: Premenopausal women (n = 25);

The normally menstruating women were included in this group; but women experiencing amenorrhea were excluded. These selected subjects were considered as control group and their age range was 25-45 years (34.52±8.03 years).

Group II: Postmenopausal women (n = 25);

Those women with one year of amenorrhea and not receiving hormone replacement therapy were considered as study group and their age range was 46-70 years (55.67±5.34 years).

Inclusion Criteria:

- Non-smokers, non-alcoholics.
- Age 25 years or more.
- No vitamins/minerals taken as supplements since last 3 months.
- Healthy females in age group 25-45 years, for premenopausal group.
- Healthy females in age group 46-70 years, for postmenopausal group.

Exclusion Criteria:

- Subjects with hypertension, cardiovascular diseases, diabetes, venereal diseases.
- Any pathology (including carcinoma).
- Women taking oral contraceptives, antioxidants or any other drug.
- Subjects with any concurrent sickness.
- Pregnancy or lactation.
- Females on hormone replacement therapy.

Sample collection

Venous blood sample was collected using aseptic techniques:

(1) 2.0 ml of fasting sample (8 –12 hrs) was collected in a plain tube for estimating estradiol hormone and Gamma GT. It was centrifuged at 1500rpm for 15min. and serum separated was stored at 4^0C.

(2) 3.0 ml of whole blood was collected in EDTA vial for the estimation of FRAP.

Biochemical analysis

The serum levels of estradiol (E2) and GGT were measured using standard kits on an auto analyser and Total Antioxidant Capacity (TAC) was determined by FRAP (Ferric reducing ability of plasma) method, which is based on the reduction of a colourless ferric tripyridyl- triazine complex to a blue ferrous complex by the antioxidants in the plasma. The change in absorbance at 593nm is directly related to the total reducing power of electron donating antioxidants present in the plasma [17].

Statistical Analysis

Statistical analysis was done, using the statistical package for social science (SPSS 20) for Windows software, Microsoft Excel 2007 and scientific calculator. The results were expressed as Mean ± Standard Deviation (SD).

Unpaired "t" tests were used to evaluate the data for the control and study groups in order to determine the levels of oxidative stress in pre and postmenopausal women as well as the differences in gamma GT levels between the two groups. The association between oxidative stress (as a measure of plasma ferric reducibility, FRAP) and levels of GGT was determined using Pearson's correlation

(r). The cut off for statistical significance was p< 0.001.

Table 1: Comparison of FRAP and Estradiol (E2) in Control and Study group

Parameter	Control group(n=25)	Study group(n=25)
FRAP (Ferric Reducing Ability of Plasma). (µmoles of $FeSO_4$ equivalent/ L of plasma)	916.08 ± 131.08	483.12 ± 53.64 *
E2 (pg/ml)	152.95 ± 60.18	18.32 ± 5.27 *

*Values in mean+SD; *p< 0.001*

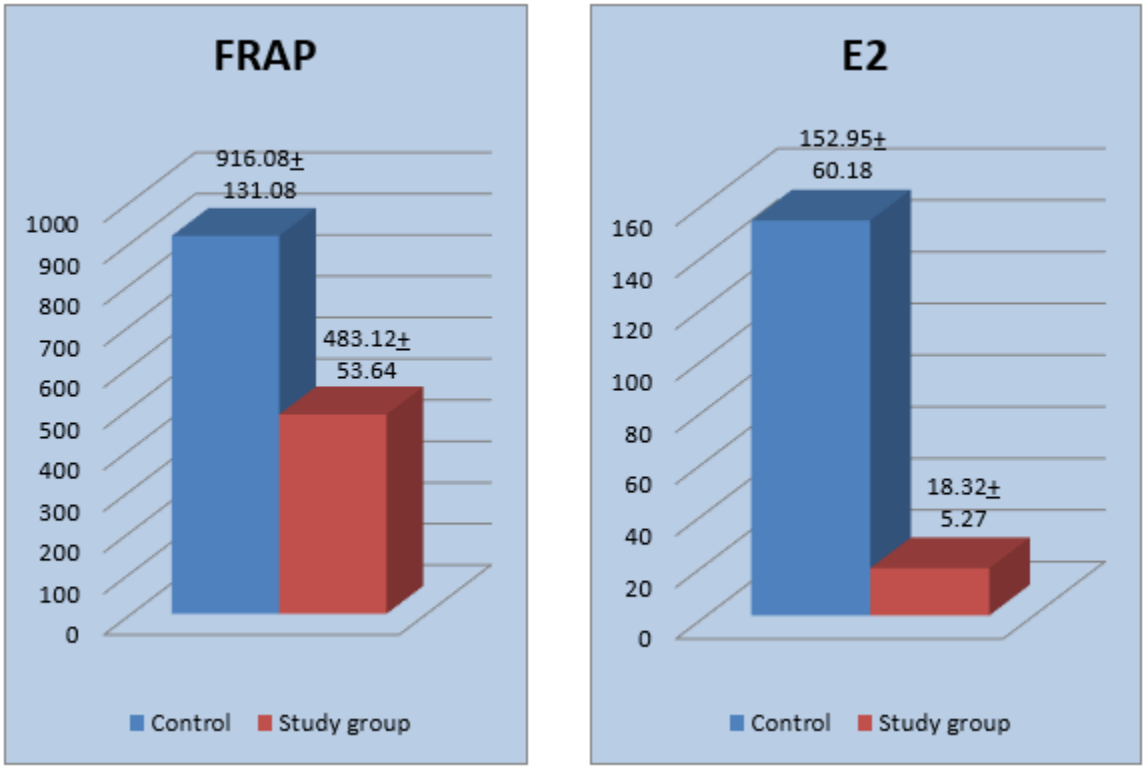

Figure 1: Comparison of FRAP and Estradiol (E2) in Control and Study group

Table 2: Comparison of FRAP and Gamma GT in Control & Study group

Parameter	Control group (n=25)	Study group (n=25)
Ferric Reducing Ability of Plasma (FRAP). (µ moles of $FeSO_4$ equivalent/ L of plasma)	916.08 ± 131.08	483.12 ± 53.64 *
γ-G T (U/L)	29.86 ± 7.73	59.87 ± 6.35 *

*Values in mean+SD; *p< 0.001*

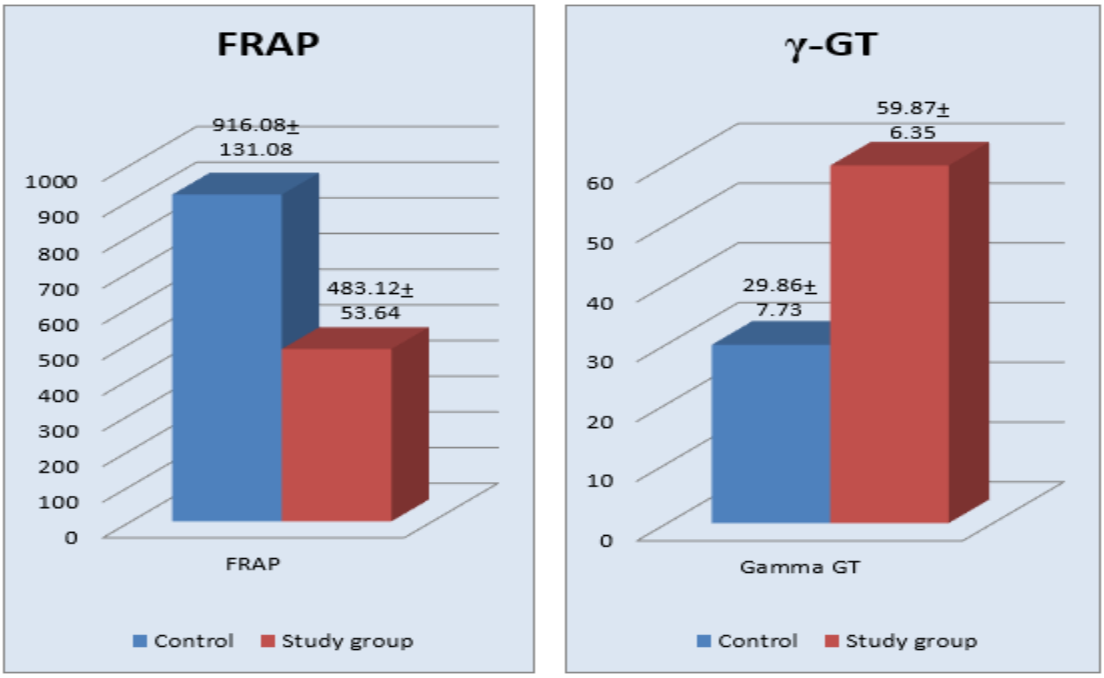

Figure 2*:* Comparison of FRAP and Gamma GT in Control & Study group

Table 3: Comparison of activity of Gamma GT and Estradiol (E2) in Control and Study group.

Parameter	Control group (n=25)	Study group (n=25)
γ- GT *(U/L)*	29.86 ± 7.73	59.87 ± 6.35 *
E2 (pg/ml)	152.95 ± 60.18	18.32 + 5.27 *

*Values in mean+SD; *p< 0.001*

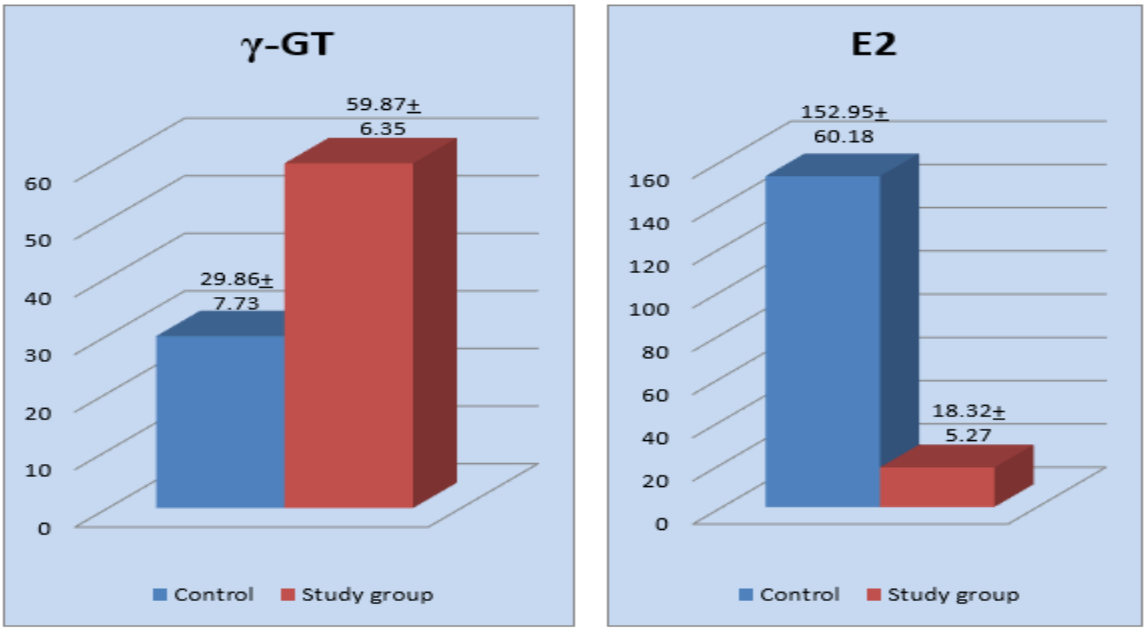

Figure 3: Comparison of activity of Gamma GT and Estradiol (E2) in Control and Study group.

Discussion

Oestrogen levels gradually decline throughout menopause. It is a normal stage of ageing, and free oxygen radicals have been suggested as significant age-related causal agents [10,11]. The results of studies cited by various authors are ambiguous when it comes to how menopause affects levels of oxidative stress. Recent research has found that oxidative stress affects a woman's entire reproductive lifespan, including the menopause [10].

Oestrogens have been shown to have free radical scavenging structures and to have in vitro antioxidant effects on membrane phospholipid peroxidation [18,19]. Free radical damage accelerates the ageing process, hence menopausal women are predicted to experience oxidative stress due to low oestrogen levels and advancing age [20,21].

In the present study the total antioxidant capacity of plasma measured as ferric reducing ability of plasma (FRAP) was reduced from 916.08+131.08 µmol/L in controls to 483.12+53.64 µmol/L of plasma in postmenopausal group, a decrease of 50% which was statistically significant ($p<0.001$). This is in concordance with the levels of estradiol (E2) that reduced drastically in menopause (Table-1, Fig. no.-1). This observation is most likely the result of depressed estrogen synthesis in postmenopausal women that enhanced oxidative stress and lead to deficit of total antioxidant capacity of plasma [22].

Reactive oxygen species (ROS) have a harmful effect on cells when they accumulate in large quantities, which makes it essential for cells to have tightly regulated metabolising and scavenging systems to get rid of them. The antioxidant enzyme Glutathione peroxidase (GSHPx) detoxifies peroxides with glutathione (GSH) acting as electron donor in the reduction reaction, producing oxidized glutathione (GSSG) as an end product [23].

Recently, it has been suggested that glutathione can provide the gamma-glutamyl residue that is transferred from gamma-glutamyl peptides to amino acids, water, and other short peptides by the enzyme known as gamma-glutamyl transferase (GGT). On the other hand, the availability of cysteine restricts the amount of glutathione that can be produced by GGT [11].

Because GGT participates in the extracellular GSH route and because GGT activity is required for the manufacture of cellular glutathione, the most significant cell antioxidant, this enzyme may be crucial to the cell's anti-oxidative defence mechanism [16]. In the present study, the mean level of Gamma GT in premenopausal women (control group) was 29.86 ± 7.73 U/L and the mean level of Gamma GT in postmenopausal women (study group) was 59.87 ± 6.35 U/L. The value of Gamma glutamyl transferase (GGT) significantly increased in case of postmenopausal women as compared to control ($P<0.001$) (Table-2, Fig.no.2).

A significant correlation between decrease in Ferric reducing ability of plasma and increase in activity of Gamma glutamyl transferase was observed. This important finding indicated that a higher level than the normal serum GGT concentration is associated with the presence of oxidative stress [24]. The decrease in estradiol (E2) levels in postmenopausal women caused by ageing and the loss of antioxidant defence mechanisms is accompanied by a significant increase in the activity of -glutamyl transferase (GGT) in these women. (Table-3, Fig. no. -3).

Conclusion

The significant increase in serum GGT in the post-menopausal group relative to the pre-

menopausal group is to compensate and correct the reduced glutathione levels by increasing its biosynthesis. Thus, GGT can be used as a marker of total antioxidant capacity of plasma and oxidative stress.

References

1. Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am. Heart J. 1986; 111: 383–390.
2. Dreisler E, Poulsen LG, Antonsen SL et al. Assessment of the endometrium in peri and postmenopausal women. EMAS Clinical Guide. 2013; 75 (2): 181– 190.
3. Baird DT, Fraser IS. Blood production and ovarian secretion rates of estradiol 17 β and estrone in women throughout the menstrual cycles. J. Clin. Endocrinol. Metab. 1974; 38: 1009. Clinical Practice. 2013: 181-203.
4. Kato I, Toniolo P, Akhmedkhanov A et al. Prospective study of factors influencing the onset of natural menopause. J. Clin. Epidemiol. 1998; 51 (12): 1271–1276.
5. Schwenkhagen A. Hormonal changes in menopause and implications on sexual health. J. Sex. Med. 2007; 3:220-226.
6. Ryan-Borchers TA, Park JS et al. Soy isoflavones modulate immune function in healthy post-menopausal women. Amer. J. Clin. Nutri. 2006: 1118-1125.
7. Gudmundsdottir SL, Flanders WD, Augestad LB. Physical activity and cardiovascular risk factors at menopause: The Nord-trøndelag health study. Climacteric. 2013; 16(4): 438-446.
8. Durackova Z. Some Current Insights into Oxidative Stress. Physiological Research. 2010; 59: 459-469.
9. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The role of oxidative stress of female reproduction: A review. Reprod. Biol. Endocrinol. 2012; 10:1-32.
10. Sohal RS, Weindruch R. Oxidatve Stress: Caloric Restriction and Aging. Science. 1996; 273: 59-63.
11. Johnson-Davis K, McMillin GA. Enzymes. In: Bishop ML, Fody EP, Schoef LE, editors. Clinical Chemistry Techniques, Principles, Correlations. 6th ed. Philadelphia: Lippincott Williams and Wilkin. 2010;300.
12. Vasudevan DM, Sreekumari S. Isoenzymes and clinical enzymology. In: Vasudevan DM, Sreekumari S, editors. Textbook of Biochemistry (for medical students). 4thed. New Delhi: Jaypee brethren's medical publishers (P) Ltd. 2005; 57.
13. Meister A. Metabolism and transport of glutathione and other ß-glutamyl compounds. In: Larsson A, Orrenius S, Holmgren A, Mannervik B, editors. Functions of glutathione: Biochemical, physiological, toxicological and clinical aspects. New York: Raven press. 1983; 1-22.
14. Włodek P, Sokołowska M, Smolensky O, Wlodek L. The β-glutamyl transferase activity and non-protein sulfhydryl compounds levels in rat kidney of different age groups. Acta Biochim Pol. 2002; 49(2):501-507.
15. Varga ISZ, Hatkovice B et al. The ferric reducing ability of plasma (FRAP). Curr Topics Biophy. 1998; 22; 219-221.
16. Abdul-Rasheed OF, Al Shamma GA, Zillo BH. Serum γ-glutamyl transferase as Oxidative Stress Marker in Pre-and Postmenopausal Iraqi Women. Oman Med. J. 2010; 25(4): 286-288.
17. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem .1996; 239: 70-76.
18. Freeman EW, Sammel MD et al. Hormones and Menopausal Status as Predictors of Depression in Women in

Transition to Menopause. Arch Gen Psychiatry. 2004; 61:62-70
19. Perz JM. Development of menopause symptoms list: A factor analysis study of menopause associate symptoms. Women and Health. 1997; 25:53 – 69.
20. Mamta M, Varun K M et al. Antioxidant status in peri-menopausal and post-menopausal women. IJABMS. 2014; 99.
21. Agarwal A, Gupta S et al. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2005; 14:3-28.
22. Sekhon LH, Agarwal A. The menopause and oxidative stress. Applied basic Research and Clinical Practice. 2013: 181 -203.
23. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother. 2003; 57(3-4):145-155.
24. Doshi SB, Agarwal A. The role of oxidative stress in menopause. J of Mid-Life Health. 2013; 4 (3):140-146.