Clinical Comparative Study for Tannic Acid and Vitamin C Activity as Antioxidants

Ra'id M. Hannun
College of Science – Thi-Qar University
thiqaruni.org

Abstract

Antioxidants are the substrates which can prevent free radicals generation and protect the cells from membrane oxidation that cause cells damage and tissue injury. In this study, we attempted to compare between tannic acid (TA) and ascorbic acid (AS) antioxidative activity *in vitro*. The study included fifty healthy subjects, and serum lipid peroxidation (LPO) was promoted by incubation with copper ions(copper sulphate).The results illustrated that the optimum concentration of copper to promote lipid peroxidation in sera was (0.1 M) with incubation at 37°C for (1 hr). The concentrations of (AS) ($10^{-5}$and 10^{-4} M) could not inhibit (LPO), whereas the concentrations (10^{-3}, 10^{-2}, 10^{-1}, 0.2) had a certain effect on (LPO) and could not decrease malondialdehyde (MDA),LPO marker, levels to the normal values with negative correlation coefficient (r= -0.72).Yet all TA concentrations(10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 0.2, 0.3)M were able to inhibit (LPO) and decrease (MDA) levels to the normal values at concentrations (10^{-1} M and up), also TA concentrations were correlated negatively with (MDA) levels (r =-0.90).

الملخص

مضادات الأكسدة هي المواد التي تمتلك القدرة على منع توليد الجذور الحرة وحماية الخلايا من عمليات أكسدة الغشاء الخلوي التي تسبب تحطيم الخلايا وتلف الأنسجة. حاولنا في الدراسة الحالية المقارنة بين الفعالية المضادة للأكسدة لكل من حامض الأسكوربيك وحامض التانيك وذلك بإجراء الاختبارات السريرية على مصل الدم المأخوذ من ٥٠ شخصاً من الأصحاء الذين شملتهم الدراسة. إذ تم حث عملية الأكسدة الفوقية للدهون في المصل بمعاملته مع أيونات النحاس (كبريتات النحاس). أظهرت النتائج بأن التركيز الأمثل للنحاس الذي ينبغي استخدامه لحث الأكسدة هو M(0.1) مع حضن لمدة ساعة واحدة في 37 °C . وجد بأن حامض الأسكوربيك بالتركيزين (M 10^{-5} ,10^{-4}) لا يمتلك القدرة على تثبيط عملية الأكسدة ، بينما استطاعت تراكيز حامض الأسكوربيك(0.2, 10^{-1}, 10^{-2}, 10^{-3}) من تقليل تركيز المالون داي الديهايد(دليل أكسدة الدهون) ولكن دون الوصول الى القيم الطبيعية له. وقد تم الحصول على علاقة ترابطية سالبة بين تركيز حامض الأسكوربيك وتركيز المالون داي ألديهايد - r =) 0.72) . بينما كانت كافة التراكيز المستخدمة من حامض التانيك(0.3, 0.2, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) قادرة على تثبيط اكسدة الدهون وتقليل مستويات المالون داي الديهايد لتصل الى القيمة الطبيعية عند استخدام التراكيز (10^{-1} M) فأعلى، كذلك فقد وجد أن تركيز حامض التانيك يرتبط بعلاقة سالبة مع مستوى المالون داي الديهايد (0.90- = r) .

Introduction

Reactive oxygen species including free radicals are naturally occurring in the body as a result of biochemical reactions during normal cellular processes (1-3). They can be increasingly formed in response to many factors such as excess pollution, too much UV sunlight, exposure to cigarette smoke, as well as inflammation processes (4-7). Additionally several *in vivo* and *in vitro*

studies had been reported that human free radicals formation processes can promoted by copper, Habber – Weiss reaction,(8-10). It is well known that the high amounts of free radicals in human body have implicated in certain chronic and aging disease such as cancers, heart disease, stroke, rheumatoid arthritis, and Alzheimer's disease(11-15). Yet free radicals generation could prevent by antioxidants that help to inhibit the many oxidation reactions caused by free radicals thereby preventing or delaying damage to the cells and tissues. Generally some antioxidants are synthesized within the cells (endogenous antioxidants) such as albumin, transferrin, ceruloplasmin…etc. While the others need to be provided in the diet (exogenous antioxidants) such as vitamins, C,E,A, and polyphenolic compounds(15,16). Natural polyphenols such as ellagic acid, tannic acid, and gallic acid are a class of phytochemicals found in high concentrations in tea, nuts, berries, cocoa, and a wide variety of other plants(18-20). The antioxidant activity of polyphenols may account for the results of a number of clinical and epidemiological studies suggesting that they may have a protective role in conditions such as cardiovascular disease.(21-26), wide variety of cancer types, antibacterial effects, and dental caries(27-29). It is known that vitamin C is a water soluble antioxidant and it is able to trap and scavenge the aqueous peroxyl radicals .Truly vitamin C has a complementary role for vitamin E, the lipid soluble antioxidant (30),.

From such a serious situation it is important to study the effects of tannic acid, one of polyphenols, on oxidation promoted by copper ions *in vitro* in this research, and to compare these effects with vitamin C activity, exogenous antioxidant,.

Design of the Study

The study was conducted in college of science in Thi-Qar University during the period from October, 20, 2005 to January, 10, 2006. The blood samples were collected from fifty healthy subjects (28 male and 22 female) aged (16-41) year.

Blood Sampling

(6 mL) of blood were withdrawn by venipuncture from each subjects at (9-11) a.m. and transferred into disposable tube. At once the samples were used in the experiments and analysis.

Materials and Methods

Blood Treatment with Copper Sulphate

Each (2 mL) of blood samples were treated with several different concentration of CuSO4 (BDH,England) (0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2) M in presence of phosphate buffer(Randox Laboratories, England) (2 mL, pH = 7.5) . The treated samples were incubated at 37 C for one hour. After centrifugation of samples for 10 min at 600 g, malondialdehyde concentration was measured in serum according to (Fong *et. al* 1973) which is described below.

Blood Treatment with Tannic Acid and Vitamin C (L-Ascorbic Acid)

After treatment of samples with copper sulphate(according to the above method), each one of them was treated with one concentration of tannic acid (Segma Co. Germany) (10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}) M .Another samples were also treated with the same concentrations of vitamin C(Segma Co. Germany). The treated samples then were incubated at 37 C for one hour. Serum malondialdehyde was measured after centrifugation of samples.

Determination of Serum Malondialdehyde

Determination of serum malondialdehyde level that consider as a lipid peroxidation marker were performed according to the method of Fong *et a1*.1973(31).In this method MDA reacts with thiobarbituric acid (TBA) (Segma Co. Germany) in shaking water bath for 90 min at 60 C to developed a colored complex MDA $(TBA)_2$ which measured at 532 nm after cooling and centrifugation for 10 min at 600 g.

Statistical Analysis

The data presented were the means and standard deviations. Correlation coefficient(r) was used to illustrate relations among certain parameters.

Results & Discussion

It is well known that free radicals and reactive oxygen species, when they formed in the body, attack the normal cells to damage them and lead to tissue injury(2). Lipid peroxidation is one of several processes which is initiated by free radicals activities on lipids of cell membranes. Therefore, in this study, malondialdehyde (MDA) (an end product of lipid peroxidation) was depended to evaluate free radicals generation and lipid peroxidation status.

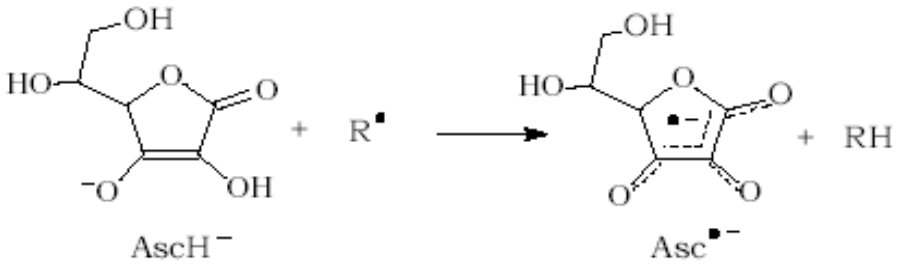
Table (1) and figure (I) showed a significant elevation in serum MDA concentration when different concentrations of copper sulphate were added to sera. However the highest elevation in MDA levels was reported at 0.1 M of CuSO4.Also the incubation time, after addition, was found to be effective on MDA level as shown in table (2) and figure (II). Truly the highest concentration

of MDA was observed after one hour of incubation at 37 C. It is notable that the MDA levels were found to increase with time of incubation.

The role of transition metals such as copper ion as a promoter of lipid peroxidation was reported in several studies and investigations (8-10). Generally, transition metals remain tightly bound to binding protein *in vivo* and are not available to participate in the oxidative biological reactions. However, in the presence of free copper ions, a sequence of two reaction steps can occur that results in hydroxyl radical (·OH) generation. In the first step, hydrogen peroxide (H_2O_2) can produce the hydroxyl radical by removing an electron from the participating copper ion. In the second step, involving the superoxide radical ($^{\cdot}O_2$), the original copper ions are regenerated so that they are again available for reaction with the hydrogen peroxide(32). This combination of two chemical reactions appears to account for most of the hydroxyl radical production in biological systems and explains, at least in part, why copper produce lipid peroxidation which assured in the present study.

The investigation of the effect of vitamin C on lipid peroxidation status *in vitro* illustrated that the mentioned vitamin has a certain role as antioxidant agent against copper mediated oxidation as shown in table (3) and figure (III).Furthermore the data in table (3) of ascorbic acid concentrations (10^{-5}, 10^{-4}, 10^{-3}) were found to be ineffective on lipid peroxidation status, serum MDA levels (51, 52, 49) nmol/mL respectively reflect this foundation,. Whereas the concentrations of ascorbic acid (10^{-2} and up) were able to decrease serum MDA levels significantly compared to the lower concentrations of it.

Vitamin C (found in two forms shown in figure IV) acts as a potent antioxidant by scavenging physiologically relevant reactive oxygen, chlorine, and nitrogen species. Several studies have shown that antioxidant nutrient supplementation, especially vitamin C was effective in protecting the lipids, proteins and DNA against oxidation *in vitro* and *in vivo*.(33-35).Generally ascorbic acid ($AscH_2$) scavenges free radicals by a known mechanism as shown below:-



AscH^{-} Asc$^{\bullet -}$

The produced radical (Asc$^{\cdot -}$) could be removed by the reaction below which represents the principal route to eliminate the Asc·- *in vitro.*As for *in vivo* it is thought that reducing enzymes are involved in the removal of this radical, resulting in the recycling of ascorbate (36)

$$2\ Asc^{\cdot -} \rightleftharpoons + H^{+} \qquad AscH^{-} + DHA$$

In this study the results assured the antioxidant role of tannic acid against copper mediated lipid peroxidation *in vitro*. Table (4) and figure (V) illustrate that all used concentrations of tannic acid have the ability to decrease serum MDA levels.

Tannic acid is a typical hydrolyzable tannin (a mixture of gallotannin figure VI). It consists of a glucose core, which covalently links to 3–5 gallic acid residues through ester bonds. In addition, each gallic acid residue can covalently link to other gallic acid molecules as shown in the mentioned figure (37). In fact this asteriated structure of tannic acid may be the essential factor by which it has the antioxidant activity(37). Furthermore the high inhibition activity of tannic acid for copper mediated lipid peroxidation might ascribe to two characteristics: the first is the chelating ability of tannic acid to transition metals including copper that promotes free radicals generation as shown above. The second is the presence of multiple phenolic groups which are able to donate their hydrogens to neutralize the free radical groups and convert them to stable molecules. The results of the present study reported that lipid peroxidation marker (MDA) correlates negatively to tannic acid concentrations with good correlation coefficient (r= -0.90), this truly demonstrates that tannic acid has an effective role as *in vitro* free radicals scavenger more than vitamin C as shown in figure (VIII). These results are compatible with the results of several studies which have demonstrated the antioxidative features of polyphenols such as tannic acid as the primary mechanism of cell rescue from membrane lipid peroxidation, and the reported values of effective polyphenols concentration in the range of (5-50 µM)(38-41).

Conclusions

Serum lipid peroxidation can be promoted *in vitro* by copper ions when incubated for 1 hr at 37 °C . Ascorbic acid has a certain role as antioxidant and it is capable of minimizing lipid peroxidation marker (serum MDA) to median levels. However tannic acid has ability to inhibit copper mediated lipid peroxidation more than ascorbic acid *in vitro* and it can minimize serum MDA to the normal value. More studies must perform for *in vivo* antioxidant activity of tannic acid in the future.

Acknowledgement

I would like to thank all students in chemistry dept. in college of science – Thi-Qar University especially Intithar Thegeel, Abeer Salih, and Aseel Sa'ad which assist me to get blood samples. Also I thank Mr. Hussain Isma'eel for research evaluation linguistically.

References

1.Freeman,B.A., and Crapo,J.D.; *Lab.Invest.* 47:412 (1982)

2.Simons,K.J.; *Am.J.Med.Assoc*.251:2187 (1984).

3.Bendich,A.; *Adv.Exp.Med.Biol*. 262:1 (1990).

4.Slater,T.F.;*Nutrition*,87:46 (1987).

5.Babior,B.M.,& Woodman,R.C.,;Semin Hematol. 27:247 (1990).

6.Murrell,G.A.C., Francis,M.I.O.,& Bromley,L.;*Biochem.J*.265:659 (1990).

7.Pryor,W.A.,Squadtio,G.L., & Friedman,M; *Free Rad.Biol.Med*.19:935 (1995).

8.Sutton,H.C., & Winterbourn,C.C.; *Free Rad.Biol.Med*.6:53 (1984).

9-Maiorino M, Zamburlini A, Roveri A and Ursini F . *FEBS Lett* 330: 174 (1993)

10- Abuja M, Albertini R and Esterbauer H . *Chem Res Toxicol* 10 10: 644(1997).

11-Emmert, D. H. and Kirchner, J. T.. *Arch. Fam. Med.*, ; 8 : 537(1999)

12- Nimata M., Okabe T.-a., Hattori, M. . Yuan, Z. Shioji, K and Kishimoto C. *Am J Physiol Heart Circ Physiol*, ; 289 : H2514 (2005).

13-Valgimigli,M. Merli,E. Malagutti,P. Soukhomovskaia,O. Cicchitelli,G. Antelli,A. Canistro,D. Francolini,G. Macri,G. Mastrorilli,F. Paolini,M. and Ferrari R. *J. Am. Coll. Cardiol.* ; 43:11: 2000 (2004)

14. Frei, B., Forte, T. M., Ames, B. N., and Cross, C. E. *Biochem. J., 277:* 133 (1991).

15. Diplock, A. T., Charleux, J. L., Crozier-Willi, G., Kok, F. J., Rice-Evans, C. A., Roberfroid, M., Stahl, W., and Vina-Ribes, J. ***Br. J. Nutr., 80:*** **S77 (1998).**

16- Jones,D.P., Eklow, L., Thor, I. I. & Orrenius; ***Arch.Biochem.Biophys*****. 210:505 (1981)**

17-. Vinson JA, . ***J Agric Food Chem;*** **43 :2800(1995)**

18. Rice- Evans CA, ***Free Rad Res;*** **2214 : 375(1995)**

19. De Whalley, ***Biochem Pharmacol*** **39:1743(1990)**

20. Yoshida H. ***Biochem Pharmacol*** **58; 1695(1999) 21- Sesso HD,.** ***Am J Epidemiol*****, 149:162(1999)**

22. Keli SO, ***Arch Intern Med,*** **156: 637 (1996)**

23. Knekt P, ***BMJ*****, 312: 478(1996)**

24. Stensvold I, ***Preventative Med,*** **21:546(1992).**

25. Yochum L, ***Am J Epidemiol*****,149: 943(1999)**

26. Hertog MGL, ***Lancet,*** **155:381(1993)**

27. Gordon MH. ***Nat Prod Rep*** **13: 265(1996)**

28. Maity S, ***Jap J Pharmacol*** **78:285(1998)**

29. Otake S,***Caries Res,*** **25:438(1991)**

30. Buettner GR. . ***Arch Biochem Biophy.*** **300:535(1993)**

31. Fong, K. L., McCay, P. B., & Poyer, J. L., ; ***J.Biol.Chem*****. 248:7792(1973)**

32. Defeng Wu, Ph.D., and Arthur I.Cederbaum, Ph.D.; ***Alcohol Research & Health*** **27:277(2003)**

33. McDowell LR. ***Vitamins in animal nutrition. Comparative aspects to human nutrition*** **. London: Academic Press,. p. 10–52 93–131(1989).**

34. Kafri I, Cherry JA. ; ***Poult Sci*****;63:125(1984)**

35. Njoku PC. ;. ***Anim Feed Sci Technol*****; 16:17(1986)**

36-Hossain MA, Asada K.. ***J Biol Chem.*** **260:12920(1985) .**

37. Stafford,H.A. Flavonoid Metabolism; CRC Press: Boca Raton,FL, p.p.65-68(1990) 38.Bastianetto S., Quirion R.; ***Neurobiol Aging*****;23:891(2002) . 39. Youdim M.B., Grunblatt E., Levites Y., Maor G., Mandel S.;*****Neurotox Res*****;4:679(2002)**

40. Choi Y.T., Jung C.H., Lee S.R., Bae J.H., Baek W.K., Suh M.H., Park J., Park C.W., Suh S.I. ; ***Life Sci*****;70:603(2001)**

41. Dore S., Bastianetto S., Kar S., Quirion R; ***Ann N Y Acad Sci*****;890:356. (1999).**

Table(1)

Effect of different concentrations of copper sulphate on serum lipid peroxidation(MDA) levels

n. of samples	$CuSO_4$ Conc. (M)	Serum MDA Conc.(nmol/mL)
5	0	13.2 ±2.1
5	0.01	20.7 ±2.8
5	0.025	27.0 ±1.9
5	0.05	38.7 ±4.1
5	0.075	46.5 ±3.3
5	0.1	45.1 ±4.7
5	0.2	52.3 ±4.1
5	0.3	51.1 ±3.8

Table(2)

Effect of incubation time on serum lipid peroxidation(MDA) levels in presence of (0.1) m of copper sulphate

n. of samples	Incubation Time	Serum MDA Conc.(nmol/mL)
5	Immediately	18.2 ±1.2
5	10 min.	29.5 ±1.4
5	15 min.	31.1 ±1.1
5	30 min.	40.2 ±1.8
5	1 hr.	49.2 ±1.5
5	2 hr.	52.7 ±1.2
5	4 hr.	51.9 ±1.9
5	8 hr.	50.1 ± 1.7

Table(3)

Effect of different concentrations of ascorbic acid on serum lipid peroxidation(MDA) levels in presence of (0.1) m of copper sulphate

n. of samples	Ascorbic Acid Conc. (M)	Serum MDA Conc.(nmol/mL)
5	0	52.3 ±3.0
5	10^{-5}	51.5 ±3.1
5	10^{-4}	52 .1±2.4
5	10^{-3}	49.8 ±1.7
5	10^{-2}	26.2 ±1.9
5	10^{-1}	27.5 ±2.7
5	0.2	25.4 ±2.1

Table(4)

Effect of different concentrations of tannic acid on serum lipid peroxidation(MDA) levels in presence of (0.1) m of copper sulphate

n. of samples	Tannic Acid Conc. (M)	Serum MDA Conc.(nmol/mL)
5	0	52.3 ±3.0
5	10^{-5}	49.2 ±2.1
5	10^{-4}	43 .1±2.7
5	10^{-3}	40.1 ±2.1
5	10^{-2}	22.7 ±2.5
5	10^{-1}	13.0 ±1.4
5	0.2	11.5 ±1.8

5	0.3	12.1 ±1.2

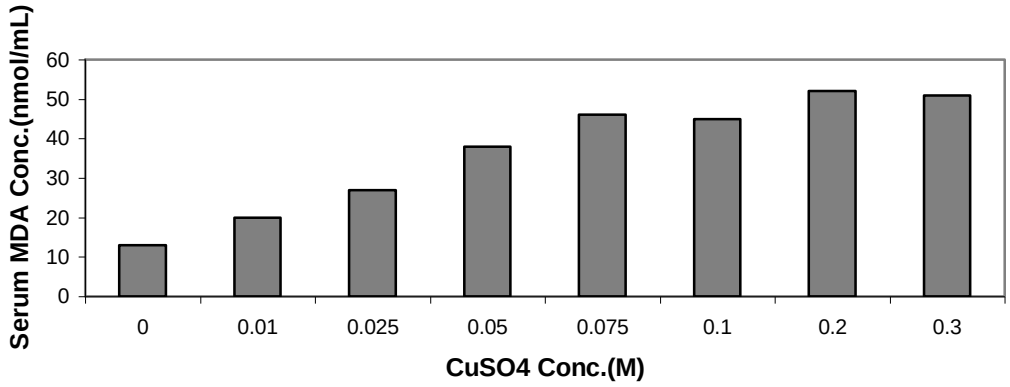


Fig.(I): Effect of Different Concentrations of Copper Sulphate on Serum MDA Levels

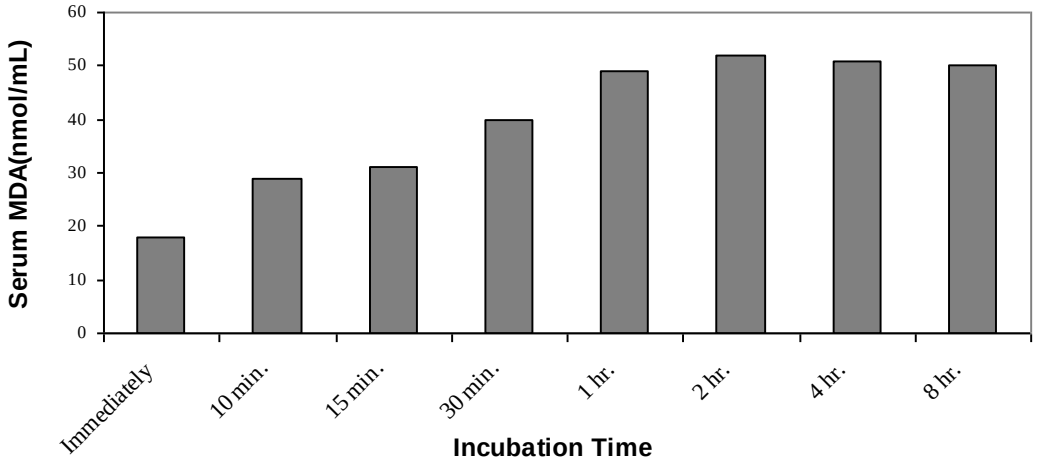


Fig.(II):Effect of Incubation Time on Serum Copper Mediated Lipid Peroxidation(MDA Levels)

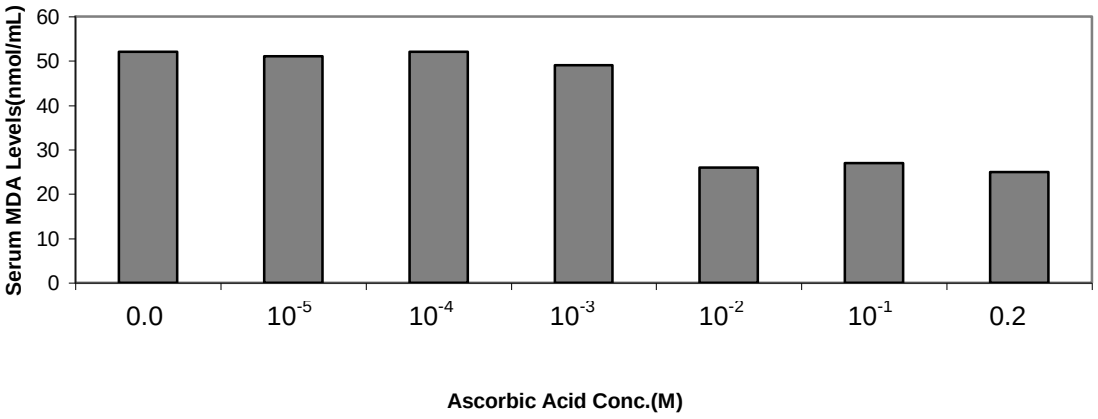
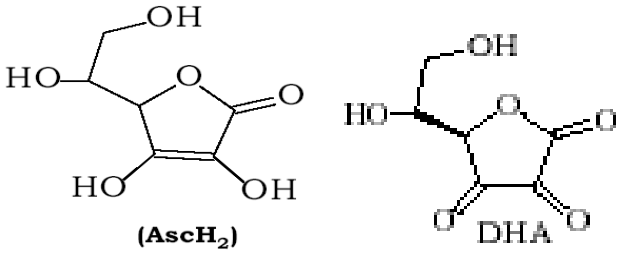


Fig.(III):Effect of Different Concentrations of Ascorbic Acid on Serum Copper Mediated Lipid Peroxidation(MDA Levels)

A **B**



($AscH_2$)

Fig.(IV) A:Ascorbic Acid Structure

B: Dehydrated Ascorbic Acid Structure

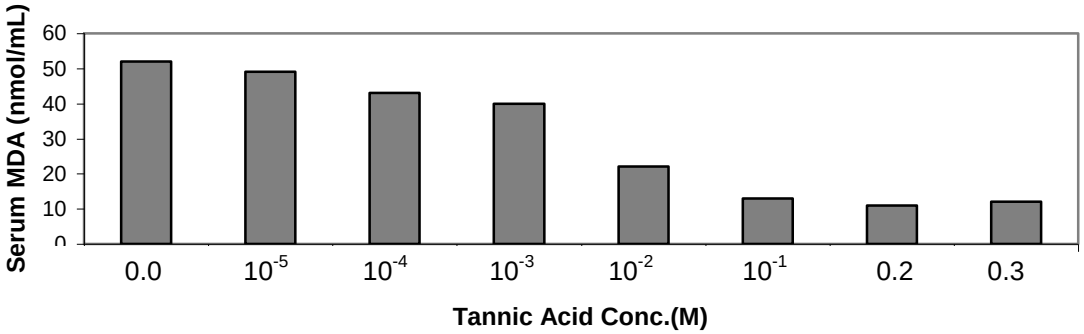


Fig.(V)Effect of Different Concentrations of Tannic Acid on Copper Mediated Lipid Peroxidation(MDA Levels)

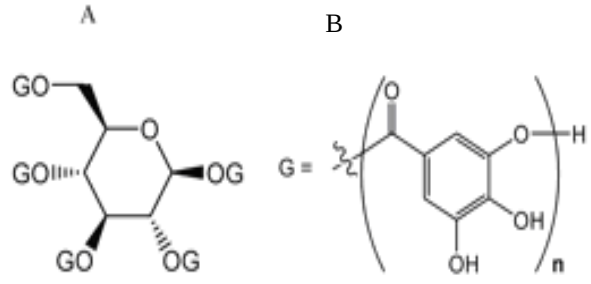


Fig.(VI)A: Tannic Acid Structure

B: Gallic Acid Structure

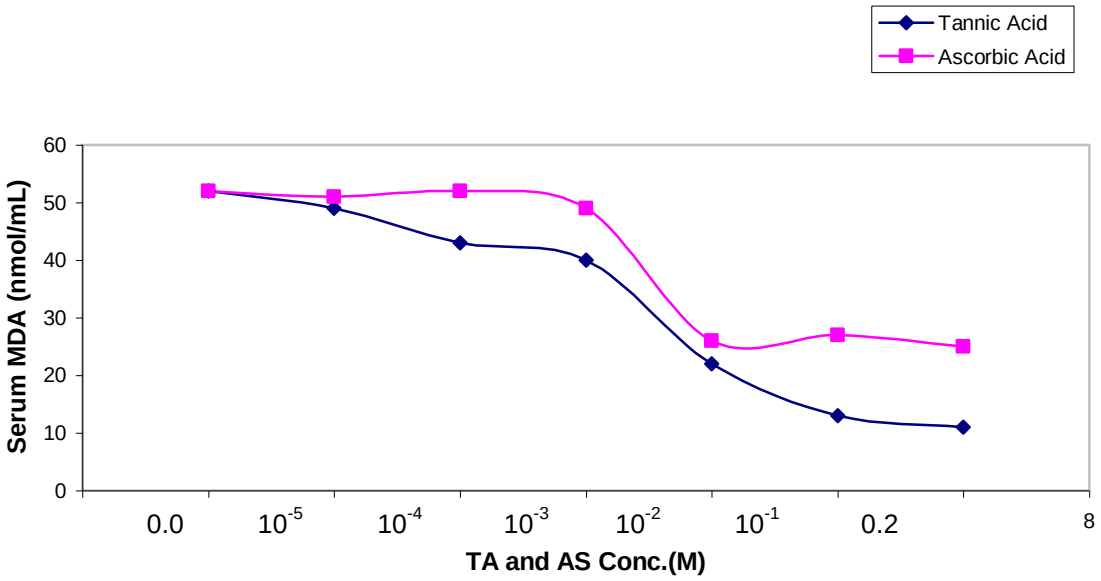


Fig.(VIII):Comparative Curve between TA and AS Effect on Serum Copper Mediated Lipid Peroxidation