STUDY THE ANTIOXIDANT EFFECT OF TOMATO EXTRACT IN OXIDATIVE STRESSED RATS.

Haider H. Humaish

Department of Pathological analysis, Technical Institute, Al-Kut Middle Technical University, Wasit, Kut

(Received 22 September 2015 ,Accepted 18 October 2015)

Keywords: Oxidative stress, GSH, Tomato extract, ALT

ABSTRACT

Tomato, is today the most popular garden vegetable in worldwide, because of its high consumption. Tomato contain a variety of phytochemical , such as lycopene , flavonoids, glycosides and other chemical constituents that have been beneficial protective effect. The present study carried out to evaluate the effect of two different doses (2 mg/kg BWand 4 mg/kg BW) of tomato extract against hydrogen peroxide-induced oxidative stress in albino rats. Thirty two adult male albino rats randomly divided into four equal groups were used in this study, the results revealed that administration of 0.75% H_2O_2 in drinking water (groupI) produced significant decline of antioxidant enzymes (superoxide dismutase (SOD) , glutathione (GSH), catalase (CAT)) and serum albumin concentration , with significant elevation of lipid peroxidation rate by estimation of malondialdehyde (MDA) and peroxynitrite radical (ONOO). Also, H_2O_2 caused significant increase in serum concentration of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), and bilirubin . On the other hand the animals treated with H_2O_2 plus 4 mg/kg BW of tomato extract (groupIII) showed significant increase of SOD, GSH, CATand albumin with significant reduction of MDA ,ONOO, ALT, AST, ALP, and bilirubin comparing with control group. Depending on the above oxidant and antioxidant markers, it seems that 4 mg/kg BW. of tomato extract exert beneficial action protect against H_2O_2 induced oxidative stress in rats.

INTRODUCTION

An increased oxidative stress has been implicated in the incidence of chronic diseases(1). The identification and study of antioxidant from dietary sourcesis a fast expanding field of research and several other important antioxidant have been investigated such as flavonoids, plant phenolics, taurine, and lycopene.Dietary intake of

tomatoes and tomato products have been associated with a decrease the risk of diseases such as cancer and cardiovascular diseases in numerous studies (2, 3). Tomato commonly used in diet, are a major sources of antioxidant and contribute to the daily intake of a significant amount of these molecules. They are consumed fresh or as processed product such as souse, juice, ketchup and soup (4). Tomato consumption lead to decreased serum lipid level and low density lipoprotein oxidation (5) .

Tomato contain a variety of phytochemicals, including carotenoids like lycopene, phytoene, provitamin A, B-carotenoid, polyphenol, including quercetin, kaempferol and other vitamin and minerals(6), Lycopene is the red color carotene found in tomato and pink grapefruit, which is a powerful antioxidant that prevent cancer and cardiovascular diseases, it also protect cell against oxidative damage caused by free radicals (4). Its contain 11 conjugated double bonds and consist of 40 carbon acyclic carotenoid .it has beneficial health effect include scavenging the single oxygen and in active free radical, modulation of intracellular gap junction communication and hormonal, immune system, and metabolic pathways have been also reported (7). Studies have indicated that lycopene is an effective antioxidant and free radical scavenger (8), because of its high number of conjugated double bond, lycopene exhibit higher single oxygen quenching ability compared to α-tocopherol or β-carotene (9) .

Free radical and reactive oxygen species (ROS), are continuously produced in the body.Free radicals are small diffusible molecules that differ from most biological molecules , they have a single electron in their outer orbital and are capable of independent existence (10) . Free radical tend to be reactive and participate in chain reactions , in which a single free radical initiation event can be propagated to damage multiple molecules (11). Several in vivo and in vitro studies have demonstrated that reactive oxygen metabolites including free radical species, superoxide anion ($O_2^{-\cdot}$) , hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$) are important mediators in tissues injury (12). therefore tissues must be protected from oxidative injury through intracellular antioxidant such as SOD , GSH, and catalase as well as extracellular (vitamin and micronutrients) antioxidant originated from herbs sources (13) . Excessive radical production and/or decreased antioxidants may lead to a condition called oxidative stress (14). Oxidative stress significantly impact multiple cellular pathways, that can lead

to the initiation and progression of varied disorder throughout the body (15), such as it can oxidize DNA and generate a large number of oxidative DNA modifications (16) and oxidative deterioration of poly unsaturated lipid that lead to lipid peroxidation (17), thus malondialdehyde (MDA) a lipid peroxidative product is used as an indicator of oxidative stress in cell and tissues. Hydrogen peroxide is one of the primary oxidants in biological system, it is lipid soluble and thus able to diffuse easily through biological membrane and reacting other cellular compartments, and induces damage to the cell membrane and decrease cell viability, furthermore, producing cellular injury especially if it become converted to the highly reactive OH (18, 19). Although almost all organism possess antioxidant defense and repair system to protect them from oxidative damage , in some cases these system are insufficient to entirely prevent such damage. Currently, there is a trend towards replacement of the widely used synthetic antioxidant with oxidant from natural sources to extend the shelf life of food and improve human health conditions (20). Fruit and vegetable are known to contain a variety of different antioxidant compound such as ascorbic acid, tochopherol , glutathione and carotenoids, which may all contribute in protection against oxidative damage. the present study was designed to investigate the phytochemical constituents of tomato extract, and evaluate the protective effect of extract against H_2O_2 induced oxidative stress in adult male rats.

MATERIALS AND METHODS

Preparation of tomato extract

Extraction of tomatoes carried out according to the method of Sadler (21). Fresh ribe tomato were obtained from local market in Al-kut city , washed well in tap water and then they were sliced with a slicer to the small pieces and then soaked in a mixture of a hexane and methanol (1: 1 v/v), and added butylated hydroxyl toluene (BHT) to minimize oxidation, in clean dark container for 24 hours with heating and shaking the mixture with shaking water bath at 38 ºC . After that the mixture was filtered properly and transfer the filtrate to a separatory funnel, and evaporate it with rotary evaporator at 45 ºC .each 1 kilogram yield 25mg of tomato extract . the extract kept in deep freezing at -4 C°.

Animal and Experimental design

Thirty two adult male albino Wister rats weighting 220 – 260 gm, aged between 3-5 months were used in this study. The rats were kept at a temperature between 22-25 C° and housed in a plastic cage (8 rats /cage) in an air conditional room, they left 2 weeks before start the study for adaptation .Control group: rats were subject *ad libiltum* supply of drinking water along the experimental period. Group I: animals of this group were given 0.75% H_2O_2 in drinking water (22) for 30 days . Group II : rats of this group were *ad Libitum* supply of0.75% H_2O_2 in drinking water, and after that they were given oral intubation 2 mg /kg.B.W. tomato extract for 30 days. Group III: Rats were given 0.75% H_2O_2in drinking water, and after that they were given oral intubation 4 mg /kg.B.W. tomato extract for 30 days.

Blood samples: blood samples were collected from each male rat via cardiac puncture using disposable syringe (5 ml). The serum was prepared by centrifugation of blood at 3000 rpm for 10 minutes and frozen at -20 C° .

Chemical assay

Serum reduced glutathione concentration (GSH) determined spectrophotometrically at 412 nm and is directly proportional to the GSH concentration (24).Antioxidant enzymes were assayed in serum. catalase activity determined by specrtrophotometric method of Goth (25).Superoxide dismutase (SOD) activitydetermined by using a modified photochemical nitroblue tetrazolium (NBT) method by using sodium cyanide as peroxidase inhibitors (26).Assessment malondialdehyde (MDA) concentration to determined lipid peoxidationBy using the thiobarbituric acid (TBA) that is read at 535nm (27).Determination of serum peroxy nitrate concentration :Peroxynitrate $ONOO^-$radical estimating was depended upon modified method of vanuffelenetal., (28) , that include nitration of phenol by peroxy nitrate radical which lead to formation nitrophenol that absorbed at 412 nm , the amount of nitrophenol is proportional to the amount of peroxynitrate radical which present in serum.

Assessment of Liver enzyme: Serum alanine aminotransferase (ALT) and Serum Aspartate aminotransferase (AST) activity were enzymatically measured using standard assay (Biomagreb chemical-kit) (29).

Serum albumin concentration: Estimation of serum albumin concentration carried out according to method of Doumasetal.(30).

Serum bilirubin concentration : Total bilirubin concentration measured spectro-photometrically at 540 nm using bilirubin standard kit (linear chemical) .

Statistical analysis : The statistical analysis of data was performed on the bases on one way analysis of variance (ANOVA) depending on the experimental design, the differences were determined using least significant differences (LSD) ($P<0.05$) (31).

RESULTS

As shown in Table (1) serum GSH, catalase and SOD were significantly ($P<0.05$) decreased in H_2O_2 treated group (Group I) with mean value 4.817 ± 0.245, 2.962 ± 0.144 and 4.065 ± 0.149 respectively as comparing to the value in the control group which were 6.935 ± 0.183 , 4.612 ±0.158, 5.345 ± 0.221 respectively , The mean value in peroxynitrite and MDA significantly ($P<0.05$) increase in the serum of rats after exposure to H_2O_2andthe values were50.89 ± 1.13 and 6.78 ± 0.173 respectively comparing to the control group (41.40 ± 1.42) and (4.32 ± 0.149) respectively,There were no significant ($P>0.05$) differences in the mean value for these parameters in Group III that treated with H_2O_2 in combination with 4 ml/kg.BW tomato extract with mean values 7.342 ± 0.219 , 3.425 ± 0.159 ,5.11 ± 0.304, 44.62 ± 1.43, 4.75 ± 0.154 for GSH, catalase, SOD, peroxy nitrate and MDA respectively as comparing with mean values of control groups.

Table (1): effect of H_2O_2 and tomato extract on serum antioxidant and serum oxidant biomarkers in rats

Test / group	GSH nmol/g	Catalase U/mg	SOD U/mg	Peroxy nitrate M/L	MDA nmol/ml
Control	**6.935 ± 0.183 A**	**4.612 ±0.158 A**	**5.345 ± 0.221 A**	**41.40 ± 1.42 A**	**4.32 ± 0.149 A**
Group I	**4.817 ± 0.245 B**	**2.962 ± 0.144 B**	**4.065 ± 0.149 B**	**50.89 ± 1.13 B**	**6.78 ± 0.173 B**
Group II	**6.452 ± 0.170 AC**	**3.556 ± 0.247 C**	**4.588 ± 0.239 BC**	**47.89 ± 1.196 BC**	**5.10 ± 0.215 C**
Group III	**7.342 ± 0.219 A**	**3.425 ± 0.159 BC**	**5.11 ± 0.304 AC**	**44.62 ± 1.43 AC**	**4.75 ± 0.154 AC**

Value are mean ± SE , n= 8 , P<0.05, capital letters denote differences between groups control : given distilled water ,Group1: given 1% H2O2 in drinking water, Group II : given 0.75 % H_2O_2 in drinking water +2 mg/kg.BW tomato extract , Group III : given 0.75 % H_2O_2 in drinking water +4 mg/kg.BW tomato extract

The result in Table (2) revealed that exposure of GroupI to 0.75% H_2O_2 daily in drinking water for 30 days led to significant (P<0.05) increase in ALT, AST, ALP and bilirubin concentration with mean value 47.46 ± 1.91 , 69.08 ± 1.42, 134.75 ± 3.49, and 1.906 ± 0.126 respectively , comparing with values of control groups of these parameters (30.79 ± 1.95), (48.09 ± 1.97), (116,75 ± 3.28) and (0.93 ± 0.061) respectively, while, treatment of animals in Group III with 4 mg/kg.BW of tomato extract showed no significant differences in the value of all previous parameter (34.16 ± 1.74), (53.90 ± 1.98),(122.95 ± 2.47) and (1.086 ± 0.097) respectively as compared with the mean values of control groups ,Thus there were no significant (P>0.05) differences between Group III and Control Group. Also , there was significant (P<0.05) decrease in the mean value of serum albumin concentration in group I that treated with H_2O_2 (1.684 ± 0.084) comparing with control group (3.008 ± 0.074) , was observed no significant (P>0.05) difference in mean value of serum albumin concentration in Group III (2.872 ± 0.080) as compared with control group.

Table (2) : Effect of H_2O_2 and tomato extract on liver enzymes and albumin & bilirubin in male rats

Test / Group	ALT IU/L	AST IU/L	ALP IU/L	Albumin mg/dl	Bilirubin mg/dl
Control	**30.79 ± 1.95 A**	**48.09 ± 1.97 A**	**116,75 ± 3.28 A**	**3.008 ± 0.074 A**	**0.93 ± 0.061 A**
Group I	**47.46 ± 1.91 B**	**69.08 ± 1.42 B**	**134.75 ± 3.49 B**	**1.684 ± 0.084 B**	**1.906 ± 0.126 B**
Group II	**40.99 ± 1.72 C**	**66.86 ± 1.78 B**	**126.28 ± 3.87 AB**	**2.038 ± 0.055 C**	**1.566 ± 0.057 C**
Group III	**34.16 ± 1.74 A**	**53.90 ± 1.98 C**	**122.95 ± 2.47 A**	**2.872 ± 0.080 A**	**1.086 ± 0.097 A**

Value are mean ± SE , n= 8 , P<0.05, control : given distilled water ,Group1: given 1**%** H2O2 in drinking water, Group II : given 0.75% H_2O_2 in drinking water +2mg/kg.BW tomato extract , Group III : given 0.75 % H_2O_2 in drinking water +4mg/kg.BW tomato extract

DISCUSSION

Glutathione is one on the essential compounds for regulation a variety of cell functions, the depletion of GSH in GroupI treated with 0.75 % H_2O_2 resulted in enhanced lipid peroxidation ,where excessive lipid peroxidation caused an increase in GSH consumption (32) . such statement was documented by Fouda (33) who revealed that inhibition of GSH level in doxorubicin- oxidative stress indicated an increase in rate transformation of GSH to oxidized glutathione (GSSG) as a result of GSH consumption to get rid of H_2O_2. This result in agreement with the some studies where reported depletion of GSH activity as a result of increase of oxidative stress (34, 35, 36). many authors reported that if the amount of ROS above the scavenging capacities of antioxidant enzymes lead to irreversible damage of cell (37). In the present study exposure of animals to the 0.75% H_2O_2 in drinking water, caused significant decreased activity of antioxidant enzymes such catalase and superoxide dismutase , these enzymes work together, catalase hydrolyzed H_2O_2 that formed via superoxide anion dismutation by activity of SOD.(37) . The study results are in concurrence with finding of

Duzgunerand Kaya (38) they reported that significant increase in hepatic lipid peroxidation and a significant decrease in hepatic antioxidants including SOD and catalase activities due to alloxan induce diabetic rats .also, the results have been shown increase malondialdehyde (MDA) and peroxy nitrate radical concentration in GroupI , comparing to the control group .

H_2O_2 is the primary oxidant on biological system and able to attack protein and lipids leading to membrane lipid peroxidation and cellular dysfunction (39). Nitric oxide that synthesized normally in the body can react with H_2O_2 to form peroxynitrite, which is potent oxidant greater than H_2O_2 alone (40).MDA is the major end product of free radical reaction on membrane fatty acids (41) . Exposure to H_2O_2 induce damage to the cell membrane and decrease cell viability, that lead to lipid peroxidation,resulting in structural alteration of membrane, loss of essential fatty acid with formation of cytosolic aldehyde and peroxide products, including MDA.

In this study, there were lack of significant differences in the mean values of Group III that treated with 4mg/kg.BW of tomato extract as compared with control group, in the above oxidant biomarkers. These results in agreement with the previous study (42) that reported tomato extract possess antioxidative properties that protect heart against adrenaline-induced myocardial infarction. Phytochemical, especially phenolics in fruit and vegetable, are suggested to be the major bioactive compounds for health benefits, the bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (43).Tomato contains a variety of phytochemicals, including lycopene, -carotene and also a variety of poly phenols such as quercetin and naringenin, which are antioxidant effects (44). Lycopene is one of the most potent antioxidant because of its high number of conjugated double bonds, exhibit higher single oxygen quenching ability compared to α- tocopherol or β-carotene (9). Several studies have indicated that lycopene is an effective antioxidant and free radical scavenger lead to decrease MDA (45,46).

Exposure of rats to the 0.75% H_2O_2 in GroupI led to increased liver enzymes comparing to the control group. Liver is the most important organ, which plays a pivotal role in regulation various physiological processes in the body. It has great capacity to

detoxicate toxic substances and synthesized useful principle (47). Hydrogen peroxide is one of the primary oxidants in biological system, it is lipid soluble and thus able to diffuse easily through biological membrane and reacting other cellular compartments, and induces damage to the cell membrane and decrease cell viability, furthermore, producing cellular injury especially if it becomes converted to the highly reactive OH (12). When liver cell membrane is damaged a variety of enzymes located normally in cytosol is released into the blood, thereby, causing an increase in level of serum ALT, AST, ALP and total bilirubin (48, 49) .The result of this study also revealed decrease of albumin concentration in H_2O_2 treated group comparing to other groups. Albumin is the most abundant plasma protein produced in hepatocytes, its depression usually reflects decrease hepatic synthesis (50) . The decrease may be due to loss of protein either by reduction protein synthesis or increase its degradation (51).The reduction of serum albumin could be attributed to changes in protein and free amino acids metabolism and their synthesis in the liver (52). Albumin has antioxidant properties due to its contain sulfhydral group, thus exposure to H_2O_2 , subsequently producing of free radical may mediated oxidation , proteolysis and degradation of albumin leading to its depression (52).

Intubation of animals with 4 mg/kg B.W. tomato extract in GroupIII improve liver function and produce reduction of liver enzymes , it may be suggested that lycopene, because of its singlet oxygen quenching ability, prevent oxidative stress of liver and heart, inhibits lipid peroxidation (reduction MDA)(53). Thus, maintaining the integrity of cell membrane of hepatocyte , subsequently decrease release liver enzyme to the blood stream and maintaining the normal values of bilirubin. Elevation of albumin concentration in tomato extract treated rats may be attributed to the minerals and antioxidant chemicals present in tomato extract that possess a powerful antioxidant activity that protect hepatocyte membrane from lipid peroxidation(54, 55).

CONCLUSION

Depending upon the antioxidant and oxidant parameters that clarified in this study and based on obtained results it seem the tomato extract has a powerful antioxidant properties that ameliorate the deleterious effect and scavenging the free radical produced

experimentally , thus, 4mg/kg B.W. is a suitable dose against experimental oxidative stress.

دراسة التأثير المضاد للتاكسد لمستخلص الطماطم ضد الإجهاد ألتأكسدي في الجرذان

حيدر حافظ هميش

قسم التحليلات المرضيه،المعهد التقني في الكوت ، واسط، العراق.

الخلاصــة

تعتبر الطماطم اكثر الخضروات شعبية في العالم وذلك نتيجة لاستهلاكها العالي . تحتوي الطماطم على العديد من المكونات الكيميائية النباتية مثل الليكوبين ، الفلافونويدات ، والكلايكوسيدات اضافة الى العديد من المواد الكيميائية الاخرى ذات التأثير الوقائي المفيد . اجريت الدراسة الحالية لتقييم تأثير جرعتين مختلفتين (2 ملغم/ كغم ، 4 ملغم / كغم من وزن الجسم) من مستخلص الطماطم ضد الاجهاد التأكسدي المستحدث بأستخدام بيروكسيد الهايدروجين بتركيز 0.75 % لدى الجرذان المختبرية. اثنان وثلاثون من الجرذان المختبرية البيضاء ، قسمت عشوائيا الى اربعة مجاميع متساوية تم استخدامها في الدراسة الحالية التي استغرقت 30 يوما. بينت النتائج بأن اعطاء بيروكسيد الهايدروجين في ماء الشرب بتركيز 0.75 % (مجموعة 1) نتج عنه انخفاض معنوي في الانزيمات المانعة للأكسدة مثل السوبر اوكساید دسميوتيز SOD ، الكلوتاثايون GSH ، الكاتاليز و تركيز الالبومين في المصل، كما اظهرت النتائج ارتفاع معنوي في معدل اكسدة الدهون من خلال تقييم المالونداياالديهايد MDA وجذر نايترات بيروكساید . كذلك يسبب بيروكسيد الهايدروجين زيادة في فعالية الانزيمات الناقلة للامين ALT ، AST ، وانزيم الفوسفاتيز القاعدي ALP والبلروبين . من جهة اخرى ، معاملة الحيوانات في المجموعة الثالثة ببيروكسيد الهايدروجين في ماء الشرب مع التجريع اليومي لمستخلص الطماطم بجرعة 4 ملغم /كغم من وزن الجسم ادى الى زيادة معنوية في SOD ، GSH ، CAT والالبومين مع انخفاض معنوي في تراكيز MDA ، ONOO ، ALT ، AST ، ALP والبلروبين مقارنة مع مجموعة السيطرة . بالاعتماد على نتائج الاكسدة ومانعات الاكسدة اعلاه . يبدو بأن 4 ملغم / كغم من وزن الجسم من مستخلص الطماطم يظهر فعالية مفيدة تحمي ضد الاجهاد التأكسدي المستحث ببيروكسيد الهايدروجين في الجرذان المختبرية .

REFERENCES

1. Janero, D. R. (1990).Malondialdehyde and thiobarbituric acid- reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free radical Biol. Med. 9:515-540.
2. Basu, A and Imrhan, V. (2007). Tomato versus Lycopene in oxidative stress and carcinogenesis: conclusion from clinical trials. *Eur. J. of clin.Nutr*. 61:295-303.
3. Roksana, P. and Nargis, A. (2008). Protective effect of tomato against adrenaline-induced myocardial infarction in rats. *Bangladesh Med. Res. Counc.Bull.*34: 104-108.
4. Bose, K.S.C. and Agrawal, B.K. (2007). Effect of lycopene from cooked tomatoes on serum antioxidant enzymes, lipid peroxidation rate and lipid profile in coronary heart disease . Singapore *Med.* 48(5):415.
5. Bub, A.; Barth, S.W.; Watzl, B.; Briviba, K. and Rechkemmer, G.(2005). Paraoxonase 1 Q192R (PON1-192) polymorphism is associated with reduced lipid peroxidation in healthy young men on a low-carotenoid diet supplemented with tomato juice. *Br. J.Nutr.* 93: 291-297.
6. Abushita, A. A.; Daood, H.G. and Biacs, P.A.(2000). Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *J.Agric. Food Chem.*, 48:2075-2081.
7. Palozza, P.; Catalano, A.; Simone, R. and Cittadini, A.(2012). A lycopene as a guardian of redox signaling. *Acta. Biochem. Pol*. 59: 21-25.
8. Trapti, G.; Purnima, D.S. and Ashok, S.(2011). Synthetic form of tomato Lycopene is effective against oxidative Stress. *Intern.J.Clin.case and Investig*. 3 : 30-37.
9. DiMascio, P.; Kaiser, S.; and Sies, H. (1989). Lycopene as the most efficient biological carotenoid single quencher .*Arch Biochem. Biophys*. 274:532-538.
10. Oluwaseun, A.A. and Ganiyu, O.(2008). Antioxidant properties of methanolic extract of *(Viscumalbum*) from cocoa and cashew trees in nageria. Afr. Biotechnol.,7:3138-3142.
11. Jones, S.; Sudweels, S. and Yakel, J.L.(2008). Radical-Free Biology of oxidative stress. Am.J.Physiol. *Cell Physiol*., 60:849-868.
12. Jay, L.Z. and Hassan T. M. (2006). The role of oxidant and free radicals in reperfusion injury. Card.V.res. 70: 181-190.
13. Kyung, M.Y.; Choong, H.L.; Hyungjae, L. Bokyung, M. and Chang, Y.L.(2008). Relative antioxidant and cytoprotective activities of common herbs. Food Chem. 160:929-936.

14. Oguzturk, H.; Polat, A.; Turtay,M.G. and Essen, B. (2010). An overview on oxidative stress parameters in emergency service workers. Stress and health, 27(3):139-142.
15. Maiese, K.; Chong, Z.Z.; Hou, J. and Shang, Y.C.(2010). Oxidative stress: Biomarkers and novel therapeutic pathways. *Exp. Gerontol*, 45: 217-234.
16. Bhattacharya, K; Alink, G. and Dopp, E. (2007). Oxidative stress and changed gene expression profile in iber particle induced carcinogenesis. *Intern. J. Human Genet.*7:1-21.
17. Beevi, S.S.; Narasu, M.L. and Gowda, B.B.(2010). Polyphenolic profile antioxidant and radical scavenging activity of leaves and stem of *Raphanussativus* L. *Plant, Foods*. Human, Nutr., 65:8-17.
18. Boveris, A.; Valdes, L.B.; Zaobomyi, T. and Bustamante, J. (2006). Mitochondrial metabolic states regulate nitic oxide and hydrogen peroxide diffusion to the cytosol. *Biochem. Biophys. Acta*., 1757: 535-542.
19. Aiyegoro, O.A. and Okoh, A.I.(2010). Preliminary Phytochemical screening and in vitro antioxidant activities of the aqueous extract of *HelichrysumLongifolium* DC. BMC Complement. *Altern. Med.,* 10:1-8.
20. Silvia, V.; Angela,A. and Stefano, M.(2004). Thr antioxidants and pro-antioxidant. Network: An overview. Current pharmaceutical Design 10(14).
21. Sadler, G.; Davis, J. and Dezman, D.(1990). Rapid extraction of lycopene and B-carotene from reconstituted tomato paste and pink grapefruit homogenates. *J. Food, Sci*. 55:1460-1461.
22. Al-Tamimi, I.A.(2011). Possible New Approach for induction of Diabetes by 0.75% hydrogen peroxide: Beneficial effects of Olive Oil. A thesis of Msc. University of Baghdad. Animal Physiology.
23. Ayako, O. M.; Fridovich, I.(2002). Subcellular distribution of superoxide dismutase in rate rate liver. *J.Biol. Chem*. 276, 38388-38393.
24. Burtis, C. and Ashwood, E.R.(1999).Tetize fundamental of clinical biochemistry. 4th Edition. W.B. Saunders company Chap 22.
25. Goth, L.(1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinic. Chimica. AC* 196:143-152.
26. Winterbournm C.C.; Hawking, R.E. and Brain, M. et al., (1975). Determination of superoxide dismutase. *J. Lab. Med*. 2:337-341.
27. Buege, J.A; and Aust, S.D.(1978). Microsomal lipid peroxidation. *Meth. Enzymol*. 51: 302-310.

28. Vanuffelen, B.E.; Van Der Zee, J.; De Koster, B.M.; Vanstevenick, J. and Elferink, J.G.(1998). Intracellular but not extracellular conversion of nitroxyl anion into nitric oxide lead to stimulation of human neutrophil migration. *Biochem. J.* 330(2): 719-722.
29. Reitman, S. and Frankle, S.J.(1957). A colorimetric method for determination of serum oxaloacetatic acid and pyruvic transaminase. Am.J.Clin. Pathol. 28:56-63.
30. Doumas, B.T; Woston, W.A. and Biggs, H.G.(1971). *Clin. Chem. Acta*. 31:87.
31. Steel, R.G.; and Terrie, J.H.(1980). Principle and procedures of statistics. A biometrical approach. 2nd Ed. McGraw-Hill Book Company. New York, USA.
32. Mohamed, H.E.; El-Swefy, S.E. and Hager, H.H.(2000). The protective effect of glutathione administration on adriamycin-induced acute cardiac toxicity in rats. *Pharmacol. Res*. 42:115-121.
33. Fouda, F.M.(2005). Anti-tumor activity of tetrodotoxin extracted from Masked Puffer fish *Arothorndiadematus. Egyp. J. of Biol*, 7:10-22.
34. Bansal, A.K.; Bansal, M.; Soni, G. and Bhatnagar, D. (2005). Protective effect of vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. *Chem Biol. Interact*. 156:101-111.
35. Pradeep, K.; Mohan, C.V.; Gobianand, K. and Karthikyan, S.(2007).Silymarin modulates the oxidant-antioxidant imbalance during diethylnitrosamine induced oxidative stress in rats. *Eur.J.Pharmacol*. 560:110-116.
36. Singh, J.P.; Selvendiran, K.; Banu, S.M.; Padmavathi, R. and Sakthisekaran, D. (2004). Protective role of Apigenin on the status of lipid peroxidation and antioxidant defense against hepatocarcinogenesis in Wister albino rats. Phytomedicine, 11:309-314.
37. Teoh-Fitzgerald, M.L. and Domann, F.E. (2012). Superoxide dismutase and cancer therapy .new York. Humana Press. Pp 59-84.
38. Duzguner, V. and Kaya, S. (2007). Effect of zinc on the lipid peroxidation and the antioxidant defense system of the alloxan-induced diabetic rabbits. Free Radic. *Biol.Med*., 42:1481-1486.
39. Rattan, S.(2006). Theories of biological aging :Genes, Proteins and Free radicals. *Free Radic.Res* 40(12):1230-1238.
40. McCord, J.M.(200**0).** The evolution of free radicals and oxidative stress. *Am.J.Med.,* 108: 652.
41. Simsek, S.; Yuce, A. and Utuk, A.E.(2006). Determination of serum malodialdehyde levels in sheep naturally infected with Dicrocoeliumdendritticum. Firat Univ. SaglikBilDergisi., 20:217-220.

42. Roksana, P. and Nargis, A. (2008). Protective effect of tomato against adrenaline-induced myocardial infarction in rats.Bangladesh, *Med. Res*. 34:104-108.
43. Mallavadhani, U,;Sudhakar, A.; Sathyanarayana, K.V.S.; Mahapatra, A.; Li, W. and Richard, B.(2006). Chemical and analytical screening of some edible mushrooms. Food Chem., 95:58-64.
44. Birt, D.F.; Hendrich, S. and Wang, W. (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol*. Then. 90:157-177.
45. Ashraf, T,A. and Usama E. M.(2012). Effect of Tomato and Guava juice on Oxidative stress in Rats after Strenuous Exercise. *J.J.Biol. Science*. 5 (3):167-174.
46. Agarwal, S. and Rao, A.V. (1998). Tomato lycopene and low density lipoprotein oxidation: a human dietary intervention study. Lipids 33:981-984.
47. Ganong, W.F.(2005). Review of Medical Physiology. 22ed McGraw Hill.Buston Barr Ridge.
48. Meganathan, M.; Madhana, G.K.; Sasikala,P.;Mohan, J.; Gowdhaman, N.; Balamurugan, K.; Nirmala, P.; Sylvia, S. and Vanitha, S.(2011). Evaluation of hepatoprotective effect of omega-3 fatty acid against paracetamol induced liver injury in albino rats. *Glob. J. of pharmacol*. 5(1); 50-53.
49. Saber, A.S.; Hoda, A.M.; and Hawazen, A.L.(2011). Protective effect of ginger (zingiberofficinale) on adriamycin-induced hepatotoxicity in albino rats. *J. of Med. Plants Res*, 5(1): 133-140.
50. Sherlock, S. and Dooly, J.(1993). Disease of the liver and biliary system. 9th Ed. Oxford: Blackwell Scientific publications, London P.17-32.
51. Swift, P.L.; Cutts, S.M.; Phillips, D.R.(2002). Activation of adriamycin by the pH-dependent formaldehyde- releasing pordrug hexamethylenetetramine. Mol. Cancer therap., 2:189-198.
52. Davies, K.J.A. and Goldberg, A.L.(1987). Oxygen radical stimulate intracellular proteolysis and lipid peroxidation by independent mechanism in erythrocytes. *J.Biol.Chem*. 262:8220-8226.
53. Ojha, S.; Goyal, S.; Sharma, C.; Arora, S.; Kumair, S. and Arya, D.S. (2013). Cardioprotective effect of lycopene against isoproterenol-induced myocardial infarction in rats. *Hum .Exp. Toxicol.* 32:492-503.
54. Dewanto, V.; Wu, X.; Adom, K.K. and Liu, R.H.(2002).Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food. Chem*. 50(10):3010-3014.

55. Malgorzata, K.; Malgorzata, E.; Teresa, A.; Czeslaw, S.; Ewa, I. and Jadwiga, J.L.(2014). Antioxidant effect of lycopene-enriched tomato paste on N-nitrosodiethylamine-induced oxidative stress in rats. *J.Physiol.Biochem*. 70 (4):981-990.