

# The Role of Green Tea Extract and Vitamin C on Malathion-Induced Testicular Oxidative Damage in Rats

اعداد

**Suzan A. Saad**

Faculty of Home Economics, Al- Azhar  
University, Nawag, Tanta, Egypt.  
[suzansaad@azhar.edu.eg](mailto:suzansaad@azhar.edu.eg)



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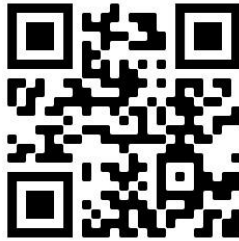
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## دور مستخلص الشاي الأخضر وفيتامين ج في الضرر التأكسدي للخصية التي يسببها الملائيون في الجرذان

د. سوزان سعد

### المستخلص

الملائيون هو أحد مبيدات الفوسفات العضوى التي تستخدم على نطاق واسع فى الزراعة لمكافحة الحشرات. يؤثر الملائيون على أعضاء الجسم كالجهاز التناسلى عن طريق تثبيط الأستيل كولينستريز وإحداث الإجهاد التأكسدى. بحثت هذه الدراسة تأثير مستخلص الشاي الأخضر وفيتامين ج فى الضرر التأكسدى (للخصية) الناجم عن الملائيون فى الجرذان. لذلك تم تصميم التجربة على النحو التالى: تم تقسيم ٤٨ من ذكور الجرذان البالغة إلى مجموعتين رئيسيتين (سته جرذان لكل مجموعة). (مج 1 : مجموعة ضابطة سلبية. مج 2 (٤٢ جرذ) تحقن بالملائيون (١٥٠ مج / كجم من وزن الجسم) فى محلول ملهى لإحداث الضرر التأكسدى للخصية. تم تقسيم المجموعة الثانية إلى سبع مجموعات فرعية ، مجموعة ضابطة موجبة ، ومجموعات تم معالجتها بفيتامين ج (١٠٠ ، ٢٠٠ مج / كجم من وزن الجسم) ومجموعات تم معالجتها بمستخلص الشاي الأخضر الكحولى والمائى (١٥٠ و ٣٠٠ مج / كجم من وزن الجسم). بعد ٢٨ يوم ، تم تخدير كل المجموعات وقياس مؤشرات الحيوانات المنوية. تم فحص الخصية لقياس الإنزيمات المضادة للأكسدة و جمع عينات من الدم لتقييم مستويات هرمونات المصل ، وهى الهرمون المنشط للحويصلة (FSH) والهرمون المنشط للجسم الاصفر (LH) والتستوستيرون. أظهرت النتائج أن المجموعات المعالجة اثرت معنويا فى حركية الحيوانات المنوية التقدمية ،الشكل الطبيعى ، إجمالى عدد الحيوانات المنوية ، هرمون التستوستيرون ، GPX ، SOD ، CAT فى المقابل ، انخفض مستوى FSH ومستوى MDA بشكل ملحوظ ( $p \leq 0.05$ ) مقارنة مع المجموعة الضابطة الموجبة. (+ ve) . خلصت الدراسة الى أن أكسدة الدهون التي يسببها الملائيون والإجهاد التأكسدى فى خصية الجرذان .يبدو أن حمض الأسكوربيك ومستخلص الشاي الأخضر بتأثيرهم المضاد للأكسدة ، يمكن أن يحسن التغيرات السامة التي يسببها الملائيون.

**الكلمات الرئيسية :** التحطم التأكسدى للخصية، الملائيون، فيتامين سى، الشاي الأخضر، مضادات الأكسدة، الهرمون المنشط للحويصلة ، الهرمون المنشط للجسم الاصفر.

## The Role of Green Tea Extract and Vitamin C on Malathion-Induced Testicular Oxidative Damage in Rats

Dr.Suzan A. Saad

### Abstract

Malathion is one of organophosphate pesticides that is widely used in agriculture to control insects. This study investigated the role of green tea extract and Vitamin C on malathion-induced testicular oxidative damage in rats. Forty eight adult male rats were divided into 2 main groups. The first group (6 rats) considered as a negative control. The second group (42 rats) administered with Malathion (150 mg/kg body weight) in saline solution to induce testicular oxidative damage. The second group were divided into seven subgroups as a following : control group (+ve), two groups treated with vitamin C (100 and 200mg/ Kg B.W.) and groups treated with alcohol or aqueous green tea extract (150 and 300 mg/ Kg BW). After 28 days, all groups were anesthetized then sperm parameters were measured. Results showed that treated groups were associated with significant increase in sperm motility (%), Progressive motility (%), Normal form, total sperm count (%), luteinizing hormone (LH) and testosterone antioxidative enzymes i.e. GP<sub>x</sub>, SOD and CAT. In contrast, follicle-stimulating hormone (FSH) and level of MDA were significantly ( $p \leq 0.05$ ) decreased as compared with (+ve) control. Malathion-induced lipid peroxidation and oxidative stress in the testis of rats. It seems that antioxidant capabilities ascorbic acid and green tea extract improved malathion-induced poisonous changes.

**Key word:** Testicular oxidative damage, malathion, vitamins C, green tea, antioxidants, sperm, LH, FSH, testosterone .

## Introduction

Diethyl methoxy thio-phosphoryl thio-succinat is a chemical pesticide Malathion organophosphate pesticide family which is widely used in industry, agriculture to control insects on crops, produce ornamental plants, grasses, fruits, and vegetables as well as in the medical sector for disease vector control in many countries.[ Dorri et al., 2015 ] These pesticides are absorbed through the skin and mucous membranes[ Sarabia and Bustos,2009 ] as affect various organs of the body including liver, kidneys, pancreas, and testis.[ Shah and Iqbal,2010 ] It inhibits acetylcholinesterase activity of most eukaryotes. The major metabolites of malathion are mono- and di-carboxylic acid derivatives, and malaoxon is a minor metabolite. However, it is malaoxon that is the strongest cholinesterase inhibitor. [ Bonner *et al*, 2007 ] oxidative stress has been lately planned as a major toxicity mechanism for organophosphorus insecticide both in acute and chronic poisoning cases [ Ranjbar *et al*, 2005 ]. Malathion affects the reproductive system. Humans, birds, and other animals are in contact to increased levels of this insecticide due to its common utilization and the rising rates of food contamination [ Babu *et al*,2006]. Testis, by producing steroids and possessing an unfortunate antioxidant group may possibly become a strong goal for the chronic oxidative stress produced during ageing [ Turner and Lysiak, 2008 ]. It is recommended that testicular oxidative stress causing dysfunction of the organ may result in infertility [ Tremellen ,2008 ].

Tea is a pleasant, common, communally accepted, and safe drink that was originally used as a medicine and is now recognized as a significant industrial and pharmaceutical raw material [ Bansal *et al*,2012 ]. Green tea polyphenols, especially epigallocatechin gallate (EGCG) have several beneficial properties, including anticancer [ Wang et al.,2018. Posadino et al.,2017 ] antioxidant, antidiabetic, antihypertensive, antimicrobial, and anti-metabolic syndrome effects [ Zhang, 2016 ] as well as improving fertility in humans and animals [ Jin et al., 2015 ]. Regular consumption of green tea is associated with a decreased risk of ovarian cancer in women [ Lee et al., 2013] Green tea is considered as a dietary source of antioxidant compounds, mainly

comprising polyphenolic components like catechins and gallic acid. Green tea also contains numerous other factors, such as vitamin C, carotenoids, and tocopherols; minerals, such as Cr, Mn, Se, or Zn; and certain phytochemical compounds [ Hashim *et al.*, 2016<sup>1</sup>. These compounds might enhance the Green tea polyphenols antioxidant activity [ Kim *et al.*, 2003<sup>1</sup>.

The ascorbic acid is a known antioxidant present in the testis with the precise role of protecting the latter from the oxidative damage [ Nayanatara *et al.*, 2003 ]. It also contributes to the support of spermatogenesis at least in part through its capacity to maintain this antioxidant in an active state. Vitamin C is itself maintained in a reduced state by a GSH-dependent dehydroascorbate reductase, which is abundant in the testes. Vitamin C has been shown to improve sperm motility and enhances semen quality and fertility of rats [Rekha *et al.* ,2009]. Deficiencies of vitamins C or E leads to a state of oxidative stress in the testes that disrupts both spermatogenesis and the production of testosterone. In recent years, vitamin C supplements have been widely used in rats diets and the levels for enhancing production and reproductive performance have been increased several fold. Supplementation with Vitamin C has also been shown to increase total sperm output and sperm concentration [ Nayanatara *et al.*, 2003<sup>1</sup>. Therefore, the present study aims to investigate the role of green tea extract and Vitamin C on malathion-induced testicular oxidative damage in rats.

## Materials and Methods

### Materials

Malathion was obtained from Kafr El- Zayat pesticide and Chemicals Company, Kafr El- Zayat, , ElGharbia Governorate, Egypt. Casein (85% protein), choline chloride, DL-methionine, vitamins and salt mixture were obtained from El-Sharqiya Company, Vitamin C was obtained from ElGomhorya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. The leaves of green tea, sunflower oil and corn starch were purchased from local market Tanta City, ElGharbia Governorate, Egypt.

## Animal

Forty- eight normal male albino rats of Sprague Dawley Strain weighing ( $150 \pm 10$ g) were obtained from the laboratory Animal Colony. Ministry of Health and Population, Helwan, Cairo, Egypt.

## Methods

### Preparation of alcohol green tea extract

Eight hundred grams of shade dried leaf powder were immersed in 4 L of 95% ethanol and left for 24 h under constant stirring and filtered. This was repeated twice with 2 L of 95% ethanol. Thus, a total of 6.5 L filtrate were collected and concentrated by rotary vapor at  $40^{\circ}\text{C}$ . The yield of ethanol extract (EE) was been 42 g (5.25%). The extraction of selected plants was done by the method of Villasenor et al.,<sup>[1]</sup> Villasenor *et al.* 2002<sup>[1]</sup>.

### Preparation of aqueous green tea extract:

The green tea extract was made by soaking amount of green tea powder which equivalent alcohol green tea extract (12 g) in boiling distilled water (100 m) five mints then solution filtered to make an effective dose<sup>[1]</sup> Maity *et al.*, 1988<sup>[1]</sup>.

## Chemical analysis

Leaves of green tea were subjected to chemical analysis in order to determine **the total phenols**: Phenolic compounds were determined by HPLC according to the method of [Goupy *et al.*, 1999] Central lab. of Food Technology Research Institute Agricultural Research Center, Cairo, Egypt.

## Experimental design and animal groups

Rats of were kept in single wire cages with wire bottoms under hygienic conditions. The diet was introduced to the rats in special food containers to avoid scattering of food. Also water supply was given *ad-libitum* and check daily. Rats fed on basal diet for one week for adaptation. After this week, the rats were divided into two main groups as a following: The first main group (**G1**= 6 rats) fed on basil diet as a negative control. The second main group administered with malathion (150 mg/kg body. weight.) to induced testicular oxidative damage and divided into

divided into seven subgroups (SG) as the following: SG (1) : was fed on basal diet as a positive control group (G+). SG (3&4) administrated orally with (100 and 200 mg vitamin C /kg body weight), respectively. SG (5&6) administrated orally with (150 and 300 mg alcohol green tea extract / kg body weight), respectively. SG (7&8) administrated orally with (150 and 300 mg aqueous green tea extract /kg body weight), respectively.

At the end of the experiment the rats were fasted overnight before sacrificed and the blood samples were collected from each rat and centrifuged to obtain the serum. Testis were collected and removed cleaned in saline solution, dried by filter paper and weighted.

### **Biological evaluation**

During the experimental period (28day), the consumed diet was recorded everyday , feed intake (FI) and body weight was recorded every week. Biological evaluation of the different diets was carried out by determination of body weight gain % (BWG %) <sup>1</sup> Chapman *et al.*,1986 <sup>1</sup>.

### **Sperm parameters**

Sperm count, Sperm motility, progressive motility and normal form were calculated according to the methods of <sup>1</sup> Ekaluo *et al.*,2005; Ekaluo *et al.*,2013 <sup>1</sup>.

### **Biochemical analysis**

#### **Hormonal assay**

The levels of hormones were measured in serum according to the principle highlighted by <sup>1</sup> Tietz, 1995 <sup>1</sup> for testosterone while the method of Uotila <sup>1</sup> Uotila *et al* 1981 <sup>1</sup> was used for luteinizing and follicle stimulating hormones.

#### **Antioxidant enzymes**

Glutathione peroxidase (GPx), Malondialdehyde (MDA), (Super Oxide Dismutase (SOD) and Catalase (CAT) were determined according to the methods of Paglia, D.E. and Valentine, Ohkawa *et al.*, Nishikimi *et al.*, and Aebi, <sup>1</sup> Paglia and Valentine , Aebi., 1984 <sup>1</sup>.



## Statistical analysis

Statistical analysis was carried out using the program of Statistical Package for the Social Sciences (SPSS), PC statistical software (Version 20; Untitled–SPSS Data Editor). The results were expressed as mean  $\pm$  Standard deviation (mean  $\pm$  SD). Data were analyzed using one way classification, analysis of variance (ANOVA) [Armitage and Berry (1987)].<sup>1</sup>

## Results and Discussion

### Chemical analysis

#### Total phenolic compounds in green tea

High-performance liquid chromatography (HPLC) analysis of green tea leaves are reported in Figure (1). HPLC analysis of leaves of green tea revealed the presence of twenty compounds in leaves of green tea. The major components are found to be catechin and chlorogenic while the lowest compounds are Alpha-coumaric and Cinnamic.

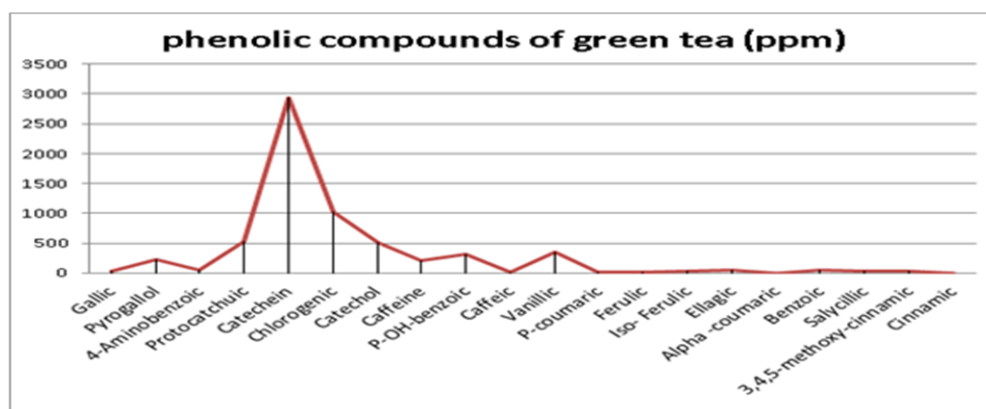


Figure (1): Phenolic compounds of green tea (ppm) by HPLC analysis

### Biological evaluation

#### Effect of green tea extracts (alcoholic & aqueous) and vitamins C on feed intake (FI), body weight gain % (BWG) and testis weight in testicular oxidative damage rats

Data presented in Table (1) showed the effect of (alcoholic and aqueous) green tea extracts and vitamins C on feed intake

(FI), body weight gain (BWG, %) and testis weight in testicular oxidative damage rats. All treated groups showed increase in FI and the best results were found in vitamin C (200 mg/kg) and aqueous green tea extract (300 mg/kg) which recorded non-significant with negative control. All treated groups recorded significant increase ( $P \leq 0.05$ ) in BWG% as compared to positive control group. The best results recorded in treated group with vitamin C (200 mg/kg). Results of testis weight recorded significant decrease in mean value of (+ve) control group as compared to all treated groups which showed improvement in testis weight when compared with (-) control group. The best results were recorded for the groups treated with Vitamin C (100 and 200 mg) and alcohol green tea extracts (300 mg/kg). Administration of aqueous extract of Green tea to pesticides cyromazine and chlorpyrifos treated groups has an ameliorated effect in the loss of body weight <sup>1</sup> Heilkal *et al.*,2013<sup>1</sup> Reddy *et al.*,2017<sup>1</sup>. Rats administered with aqueous green tea extract showed the observed testis weight loss in (+ve) control group may be due to reduced sex hormones <sup>1</sup> Nashwa *et al.*,2011<sup>1</sup>

**Table 1:** Effect of green tea extracts (alcoholic & aqueous) and vitamins C on feed intake (FI), body weight gain % (BWG) and testis weight in testicular oxidative damage rats

Parameters Groups	FI (g)	BWG (%)	Testis weight (g)
Control -ve	252±2.0 <sup>a</sup>	34.916±1.24 <sup>a</sup>	2.27±0.02 <sup>a</sup>
Control +ve	240±2.0 <sup>d</sup>	19.84±1.16 <sup>f</sup>	1.75±0.08 <sup>e</sup>
Vitamin C 100 mg	244±2.0 <sup>c</sup>	25.19±1.00 <sup>c</sup>	2.21±0.02 <sup>abc</sup>
Vitamin C 200 mg	250±2.0 <sup>ab</sup>	29.63±1.10 <sup>b</sup>	2.25±0.02 <sup>ab</sup>
Alcohol green tea extract 150 mg	244±1.0 <sup>c</sup>	21.57±1.15 <sup>e</sup>	2.14±0.02 <sup>cd</sup>
Alcohol green tea extract 300 mg	247±1.0 <sup>bc</sup>	22.62±0.68 <sup>de</sup>	2.25±0.02 <sup>a</sup>
Aqueous green tea extract 150 mg	246±2.0 <sup>c</sup>	22.68±0.53 <sup>de</sup>	2.11±0.04 <sup>d</sup>
Aqueous green tea extract 300 mg	250±2.0 <sup>ab</sup>	23.69±0.79 <sup>cd</sup>	2.18±0.04 <sup>c</sup>

\*Values denote arithmetic means ± SD. Means with different letters in the same column differ significantly at  $p \leq 0.05$ .

## Effect of green tea extracts (alcoholic & aqueous) and vitamins C on the sperm parameters in testicular oxidative damage rat

Data listed in Table (2) declared that (+ve) control group showed a significant reduction in Sperm motility (%) as compared to all treated groups. The best result was recorded for the group treated with alcohol green tea extracts (150 and 300 mg/kg) and Vitamin C (200 mg) which recorded non- significant with negative control. As for Progressive motility (%) and Normal form (%) it could be observed that experimental groups treated with green tea extracts (alcoholic & aqueous) and vitamins C had noticed improvement which recorded increase in Progressive motility (%) and Normal form (%). The best results were recorded for the groups treated with Vitamin C (200 mg) and alcohol green tea extract (300 mg/kg).

The same table illustrate the change in total sperm count for controls and treated groups. Results of total sperm count showed that all treated groups had significant increase as compared to (+ve) control group. The best result recorded for the groups treated with alcohol green tea extract (300 mg/kg). Vitamin C can be found in high concentrations in seminal plasma [Larson *et al.*, 2003; Jouanne *et al.* 1988] As vitamin C intake increases its concentration in seminal plasma rises and prohibits DNA damage [Douglas *et al.*, 2005] Administration of green tea improve sperm parameter because green tea significantly increases the antioxidant capacity in plasma as well as spermatozoa after consumption of 2–6 cups/day which may lead to decrease oxidative damage of lipids and DNA [Henning *et al.*, 2003, Xu *et al.*, 2004, Higdon and Frei, 2003]. Green tea increases the antioxidation level and protects against oxidative damage in humans. Therefore, green tea regulates defensive mechanisms against oxidative damage [Erba *et al.* 2005]. In addition to green tea antioxidant properties, it's may also decrease inflammation, reduce DNA fragmentation, and increase the motility and viability of semen [Asadi *et al.*, 2017]. Other potential benefits of polyphenols of green tea include improved egg viability and reduced cellular damage of reproductive organs.

In addition, its antioxidative concentration correlates with sperm levels and motility [ Sreejaya and Nirmala 2016 ]<sup>1</sup>.

**Table 2:** Effect of green tea extracts (alcoholic & aqueous) and vitamins C on the sperm parameters in testicular oxidative damage rat

Parameters Groups	Sperm motility (%)	Progressive motility (%)	Normal form (%)	Total sperm count (10 <sup>6</sup> )
Control -ve	66.67±2.89 <sup>a</sup>	56.67±3.51 <sup>a</sup>	68.33±2.89 <sup>a</sup>	32.52±0.80 <sup>a</sup>
Control +ve	38.67±3.06 <sup>d</sup>	33.33±2.89 <sup>c</sup>	36.00±3.61 <sup>f</sup>	17.49±1.25 <sup>e</sup>
Vitamin C 100 mg	56.67±2.89 <sup>bc</sup>	46.33±3.21 <sup>b</sup>	57.33±2.52 <sup>cd</sup>	23.25±0.73 <sup>d</sup>
Vitamin C 200 mg	61.00±3.61 <sup>ab</sup>	48.33±2.89 <sup>b</sup>	66.00±3.61 <sup>ab</sup>	26.34±1.34 <sup>c</sup>
Alcohol green tea extract 150 mg	61.67±3.51 <sup>ab</sup>	44.67±2.52 <sup>b</sup>	56.33±1.53 <sup>d</sup>	25.66±0.69 <sup>c</sup>
Alcohol green tea extract 300 mg	64.67±2.52 <sup>a</sup>	54.00±3.61 <sup>a</sup>	63.33±2.89 <sup>ab</sup>	29.64±0.69 <sup>b</sup>
Aqueous green tea extract 150mg	51.67±2.89 <sup>c</sup>	43.33±3.51 <sup>b</sup>	50.33±2.52 <sup>e</sup>	24.52±1.26 <sup>cd</sup>
Aqueous Green tea extract 300mg	58.00±3.46 <sup>b</sup>	46.00±3.61 <sup>b</sup>	61.67±2.89 <sup>bc</sup>	25.65±0.93 <sup>c</sup>

\*Values denote arithmetic means ± SD. Means with different letters in the same column differ significantly at p≤0.05.

## Biochemical analysis

### Hormonal assays

Data found in the Table (3) declared that LH (mIU/mL) and testosterone (ng/ml) in all treated groups with green tea extracts (alcoholic & aqueous) and vitamins C recorded significant increase as compared to positive control group. The best results in LH and testosterone reported in the group treated with Vitamin (C 200 mg) which recorded the nearest mean value from normal control group.

As for FSH (mIU/mL) it could be observed that positive control group achieved mean value higher than negative and all treated groups with significant difference between them. The best result founded in the group treated with Vitamin (C 200 mg) which recorded the nearest mean value from normal control group.

Testosterone is produced primarily in the gonads under the influence of the pituitary FSH and LH. The observations made from the results of this study therefore suggested that malathion the potential to interact and disrupt the functionality of the gonadal tissues in male rats. In our study, vitamin C administration to rats exposed to malathion produced an appreciable increase in the serum testosterone level to the level within the range of the control level and prevention of the alteration of the architectural integrity of the testicular tissues. These observations gave an indication that vitamin C counteracted the adverse effects of the constituents of malathion on the gonadal tissues in male rats. The result of this study agrees with the previous reports that vitamin C is an effective antioxidant in various biological systems [Ayo *et al* 2006 Ambali *et al.*, 2007, Chen *et al.*, 2000, Frei, 2004].

**Table 3:** Effect of green tea extracts (alcoholic & aqueous) and vitamins C on Luteinizing hormone (LH), Follicle-stimulating hormone (FSH) and Testosterone in testicular oxidative damage rats

Parameters Groups	LH (mIU/mL)	FSH(mIU/mL)	Testosterone (ng/ml)
Control -ve	2.87±0.08 <sup>a</sup>	2.79±0.03 <sup>g</sup>	2.91±0.03 <sup>a</sup>
Control +ve	1.42±0.04 <sup>f</sup>	5.33±0.06 <sup>a</sup>	1.82±0.05 <sup>f</sup>
Vitamin C 100 mg	2.54±0.04 <sup>d</sup> <sup>e</sup>	3.06±0.05 <sup>d</sup>	2.52±0.02 <sup>e</sup>
Vitamin C 200 mg	2.71±0.05 <sup>b</sup>	2.89±0.04 <sup>f</sup>	2.82±0.02 <sup>b</sup>
Alcohol green tea extract 150 mg	2.45±0.05 <sup>e</sup>	3.18±0.04 <sup>c</sup>	2.48±0.03 <sup>e</sup>
Alcohol green tea extract 300 mg	2.63±0.07 <sup>bc</sup>	2.98±0.03 <sup>e</sup>	2.70±0.04 <sup>c</sup>
Aqueous green tea extract 150 mg	2.45±0.04 <sup>e</sup>	3.30±0.03 <sup>b</sup>	2.59±0.04 <sup>d</sup>
Aqueous green tea extract 300 mg	2.60±0.03 <sup>cd</sup>	3.10±0.05 <sup>d</sup>	2.66±0.03 <sup>c</sup>

\*Values denote arithmetic means  $\pm$  SD. Means with different letters in the same column differ significantly at  $p \leq 0.05$

Green tea extract indicate stimulation in testosterone synthesis in testes by components of green tea extract which increase the activity of enzymes which are responsible for synthesis and metabolism of leydig's cells steroids by stimulation of interstitial cells stimulating hormone (ICSH) which convert cholesterol to pregnonolone and then testosterone. Also spermatogenic stimulating hormone (FSH) or (SSH) play a role in testosterone synthesis by increasing receptors sensitivity to (LH) or (ICSH) which found on the surfaces of leydig's cells and then increase steroidogenesis and testosterone release in the seminiferous tubules [ Pineda *et al.*, 2006 ]<sup>1</sup>

### Antioxidant enzymes

Glutathione peroxidase (GPx), Super Oxide Dismutase (SOD) and Catalase (CAT) were determined as important endogenous antioxidant enzymes in the testis. As can be seen from Table 4, the malathion-treated group has MDA levels that were substantially increased ( $p < 0.05$ ), and their GPx, SOD and CAT levels were significantly reduced ( $p < 0.05$ ). Results of GPx, SOD and CAT showed that all treated groups recorded significant increase when compared with positive control group. On the other hand, all treated groups recorded significant decrease in MDA when compared with positive control group.

**Table 4:** Effect of green tea extracts (alcoholic and aqueous) and vitamins C on antioxidant enzymes (GP<sub>x</sub>, SOD, CAT and level of MDA) of testicular tissue in testicular oxidative damage rats

Parameters Groups	GPX ng/mg	SOD U/L	CATng/mg	MDA nmol/mg
Control -ve	0.40 $\pm$ 0.05 <sup>a</sup>	0.34 $\pm$ 0.03 <sup>a</sup>	0.31 $\pm$ 0.04 <sup>a</sup>	0.14 $\pm$ 0.01 <sup>d</sup>
Control +ve	0.18 $\pm$ 0.02 <sup>d</sup>	0.19 $\pm$ 0.02 <sup>d</sup>	0.19 $\pm$ 0.02 <sup>c</sup>	0.28 $\pm$ 0.02 <sup>a</sup>
Vitamin C 100 mg	0.30 $\pm$ 0.01 <sup>b</sup>	0.28 $\pm$ 0.01 <sup>bc</sup>	0.27 $\pm$ 0.02 <sup>ab</sup>	0.19 $\pm$ 0.01 <sup>bc</sup>
Vitamin C 200 mg	0.37 $\pm$ 0.02 <sup>a</sup>	0.32 $\pm$ 0.06 <sup>ab</sup>	0.30 $\pm$ 0.04 <sup>a</sup>	0.16 $\pm$ 0.02 <sup>d</sup>

Alcohol green tea extract 150 mg	0.25±0.04 <sup>c</sup>	0.28±0.01 <sup>bc</sup>	0.28±0.01 <sup>ab</sup>	0.19±0.01 <sup>bc</sup>
Alcohol green tea extract 300 mg	0.31±0.02 <sup>b</sup>	0.31±0.02 <sup>a</sup>	0.30±0.01 <sup>a</sup>	0.17±0.02 <sup>cd</sup>
Aqueous green tea extract 150 mg	0.30±0.01 <sup>b</sup>	0.25±0.02 <sup>c</sup>	0.24±0.01 <sup>b</sup>	0.21±0.02 <sup>b</sup>
Aqueous green tea extract 300 mg	0.31±0.01 <sup>b</sup>	0.31±0.01 <sup>ab</sup>	0.28±0.01 <sup>a</sup>	0.16±0.01 <sup>d</sup>

\*Values denote arithmetic means ± SD. Means with different letters in the same column differ significantly at  $p \leq 0.05$ .

Pesticides by making some changes in DNA or its binding protein can damage the testis tissues and cause mutations in spermatogonia cells, which ultimately lead to changes in the sperm.<sup>[Ogutcu et al., 2006]</sup> And also, it is proved that any increase in the levels of MDA and reducing the antioxidant immune system.<sup>[Jahromi et al., 2012]</sup> In such circumstances, along with avoiding exposure to organophosphate pesticides, use of an appropriate antioxidant such as ascorbic acid used in this research can reduce adverse effects of exposure to these pesticides. Vitamin C is available in many foods and easily intake an antitoxic effect by daily consuming<sup>[Moron et al 1979]</sup> Ascorbic Acid is a water-soluble vitamin that can decrease the amount of free radicals through its antioxidant properties<sup>[Sutcu et al 2006]</sup>.

Antioxidative enzymes are activated by green tea extracts intake<sup>[Frei and Higdon 2003]</sup>, and the antioxidative strength of human plasma increases with continual ingestion of green tea<sup>[Kimura et al 2006]</sup>,<sup>[These antioxidative defense systems might also prevent oxidative damage in the brain. Long-term intake of green tea extracts may be important because cells are constantly exposed to oxidative stress [Khan et al 1992].</sup>

## Conclusion

The present study data demonstrate that malathion can produce adverse effects on fertility, reproductive performance, and sperm parameters in male rats. In addition, it seems that

ascorbic acid and green tea extract due to its antioxidant capabilities, can improve malathion-induced poisonous changes.

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