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# Efficacy of Clove Oil as an Antioxidant against Radiation Risk in Male Rats

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# ABSTRACT

The present study was undertaken to evaluate the antioxidant capacity of clove oil (150 mg/kg b.wt) orally administered for 21 consecutive days before irradiation of male rats. Animals were divided into four groups, control rats group, treated rats group, irradiated rats group and treated irradiated rats group. Blood samples were collected 1 and 7 days post irradiation. Exposure of rats to gamma irradiation (6.5Gy) induced significant decrease in glutathione (GSH) content, the activity of superoxide dismutase (SOD), and catalase activities, leutinizing hormone (LH) and testosterone hormone levels in blood while significant elevation in MDA level, total cholesterol level (TC), triglycerides level (TG), low density lipoprotein-cholesterol level (LDL) and follicle stimulating hormone (FSH) was recorded. The data obtained from rats treated with clove oil before whole body gamma irradiation revealed an improvement in antioxidants enzymes activities and concentration of GSH associated with significant reduction of blood MDA concentration, comparing with irradiated rats. Moreover, significant amelioration in the total cholesterol, triglycerides, and FSH, LH and testosterone hormones was recorded. It could be concluded that clove oil exerts a beneficial protective role against radiation induced oxidative stress and some biochemical disorders.

*Key words: clove oil, antioxidant, lipid, hormones, rats, γ-rays.* 

# **INTRODUCTION**

Radiation injury to living cells is too a large extent, due to oxidative stress <sup>(1)</sup>. Ionizing radiation causes a variety of changes in tissues depending on the radiation dose, duration of exposure and the susceptibility of tissues to ionizing radiations <sup>(2)</sup>. Antioxidants such as glutathione, arginine, citrulline, taurine, creatine, selenium, zinc, vitamin E, vitamin C, vitamin A and tea

polyphenols and antioxidants enzyme such as superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase exert synergistic actions in scavenging free radicals <sup>(3)</sup>.

Many natural antioxidant, whether consumed before or after radiation exposure, are able to confer some levels of radio protection<sup>(4)</sup>. Spices have been used as food seasoning and for medicinal purposes for centuries. Several of them possess hypolipidemic, antiplatelet aggregation, anti tumor and immune stimulate activities. These pharmacological properties may be useful to reduce the risk of cardiovascular disease and cancer. This is due to the presence of phenolic, polyphenolic, terpenoids and carotenoids<sup>(5)</sup>.

Traditionally clove has been used as a spice worldwide <sup>(6)</sup>. Clove oil is an essential oil from the dried flower, buds, leaves and stems of the tree *Syzygium aromaticum* (Eastern Henisphere ) or *Eugenia caryophyllate* and *Eugenia aromaticum* (western Hemisphere) <sup>(7)</sup>. This oil is in the myrtle family (Myrtaceae). A key constituents is Eugenol-75-87 % which is a phenol making this very potent oil <sup>(6)</sup>.

Clove has been reported to possess a potent antioxidant activity in vitro<sup>(8)</sup>, which reduces the oxidative stress in the body <sup>(9)</sup>. Many investigators demonstrated the hepatoprotective properties of clove against chemical stress <sup>(10)</sup> as against physical stress <sup>(11)</sup>. Clove is reported as aphrodisiac <sup>(12)</sup>, stomachic <sup>(13)</sup>, carminative, and antispasmodic <sup>(14)</sup>. It is reported to act as a contraceptive in low doses<sup>(15)</sup> and useful in cataract<sup>(16)</sup>, have anticarcinogenic property<sup>(17)</sup>. Moreover, clove inhibits platelet aggregation and alters arachidonic acid metabolism in human platelets <sup>(18)</sup>. The main constituents of the essential oil of clove are phenylpropanoids such as carvacol, thymol, eugenol and cinnaldehyde <sup>(19)</sup>. The biological activity of clove oil has been investigated in several purposes, including antioxidant properties (20), free radical scavenging (21), metal chelation<sup>(22)</sup>, neuroprotection<sup>(23)</sup>, antidiabetic<sup>(24)</sup>, antibacterial and insecticidal properties<sup>(19)</sup>, gastroprotective activity<sup>(25)</sup>. Eugenol inhibits lipid peroxidation and maintains activities of enzyme superoxide dismutase, catalase, glutathione peroxidase and glucose-6 phosphate dehydrogenase<sup>(26)</sup>. The lethal clove oil dose is 3.752 g/kg body weight  $^{(27)}$ .

The present study has been conducted to investigate the hazards effects of exposure to whole body gamma irradiation on male albino rat and to evaluate the possible protective role of clove oil, this included the effect on antioxidants (catalase activity, superoxide dismutase activity, glutathione content) and malondialdehyde level, total cholesterol, triglycerides low density of lipoprotein-cholesterol as well as follicle stimulating, leutinizing and testosterone hormones.

#### MATERIALS AND METHODS

- **Experimental animals:** Adult male albino rats weighting 120-150 gm were obtained from the Egyptian organization for biological product and vaccines and kept under normal conditions of pressure, ventilation, temperature and humidity. Animals were fed on standard pellet diet
- **Radiation exposure**: The source of radiation was gamma cell 40 (cesium-137) at the National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt. The dose rate at the time of experiment was 0.61 Gy/min. Rats were irradiated with a single dose level of 6.5 Gy.
- Experimental design: Animals were divided into four equal groups (n=12).
- Group 1: Control. Animals of this group didn't receive any treatment.
- **Group 2:** Clove oil. Animals of this groups received clove oil orally via stomach tube at a dose level 150mg/kg body weight/day for 21 consecutive days.
- **Group 3:** Irradiated. Animals of this group were whole body exposed to gamma irradiation (6.5 Gy) as a single dose.
- **Group 4:** Clove oil + Radiation. Animals of this group received clove oil supplementation for three weeks (150 mg/kg b.wt), then exposed to whole body gamma irradiation.

Animals were sacrificed 1 and 7 days post irradiation. Blood samples were obtained by heart puncture then divided into 2 parts. The 1<sup>st</sup> part was collected on EDTA to measure glutathione content (GSH), superoxide dismutase (SOD) and catalase activities. Glutathione content was determined according to Beutler, *et al.* <sup>(28)</sup>. The activity of superoxide dismutace (SOD) and catalase was determined according to the methods of Minami and Yoshikawa,<sup>(29)</sup> and Bergmeyer and Grabe,<sup>(30)</sup>, respectively. The lipid peroxide malonaldehyde (MDA) content was determined according to Yoshioka et al., <sup>(31)</sup>.

The 2<sup>nd</sup> part of the blood was allowed to clot and centrifuged at 3000 rpm for 10 min and the collected serum was stored in a deep freezer for estimation luteinizing hormone (LH) and follicle stimulating hormone (FSH) according to Garrett, <sup>(32)</sup>, Estimation of testosterone hormone was performed according to the method of Wilson and Foster, <sup>(33)</sup>, Serum total cholesterol, Low

density lipoprotein cholesterol (LDL) and triglycerides(T.G.) were determined according, to Allain *et al.* <sup>(34)</sup>, Demacker *et al.* <sup>(35)</sup> andFossati and Prencipe, <sup>(36)</sup>, respectively.

Data were statistically analyzed using a computer program for student ttest according to Snedecor and Cochern, <sup>(37)</sup>.

## RESULTS

The oral administration of clove oil (150 mg/kg b wt) for 21 consecutive days induced non-significant changes in GSH, SOD, catalase, MDA, cholesterol, triglycerides, LDL, FSH, LH and testosterone (Tables 1, 2 and 3).

As shown in Table (1) the results obtained from rats exposed to single whole body irradiation (6.5Gy) are characterized by significant decrease in blood GSH content, catalase activity and SOD activity and is accompanied by significant increase in blood MDA level, 1 and 7 days post exposure, compared to control group. Supplementation of clove oil to rats pre-irradiation has significantly increased GSH content, catalase activity and SOD activity and significantly decreased MDA level 1 and 7 days post irradiation, compared to irradiated group.

 Table (1): Effect of clove oil (150mg/kg b.wt) on blood glutathione (GSH) content, superoxide dismutase (SOD) catalase activity and malondialdehyde (MDA) level in different animal groups.

Groups	Days	GSH (mg/dl)	SOD (U/ml)	Catalase (U/ml)	MDA (mmol/L)
Control	1 <sup>st</sup>	55.23±2.48	6.59±0.22	50.05±2.56	161.90±1.73
	7 <sup>th</sup>	56.56±2.73	6.61±0.18	51.31±2.61	163.062±1.59
Clove oil	1 <sup>st</sup>	56.98±2.06 3.17%	6.71±0.21 1.82	51.20±2.40 2.30	159.23±2.71 -1.65
% change	7 <sup>th</sup>	54.81±1.60 3.09	6.18±0.18 -6.51	53.14±2.92 3.57	161.01±2.14 -1.26
Irradiated	1 <sup>st</sup>	40.32±2.08* -27	5.32±0.12* -19.27	42.35±1.03* -15.38	184.38±3.67* 13.89
% change	7 <sup>th</sup>	38.07±1.68* -30.54	5.18±0.15* -16.18	40.42±2.83* -23.93	186.25±2.81* 15.68
Cove oil +Irradiation	1 <sup>st</sup>	53.55±1.25# -6.02	6.34±0.15# -5.51	47.54±2.64# -7.15	164.74±3.60# 3.46
% change	7 <sup>th</sup>	51.99±2.25# -8.08	6.10±0.18# -7.72	51.56±2.66# 0.49	162.29±2.82# -0.47

The % change of control.

Each value represents the mean of 6 observations ± S.E.

\* Significantly different from control values.

# Significantly different from the values of irradiated rats.

Table (2) showed a significant increase in cholesterol, triglycerides and LDL levels after exposure to irradiation (6.5 Gy) on the 1<sup>st</sup> and 7<sup>th</sup> day, compared to control group. Significant amelioration was observed in animals orally administered clove oil for 21 days pre- exposure to irradiation, compared to irradiated group.

Groups	Days	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)
Control	1 <sup>st</sup>	54.34±0.61	102.74±1.50	27.82±0.30
	7 <sup>th</sup>	55.14±0.81	103.84±1.60	27.62±0.19
Clove oil	1 <sup>st</sup>	55.22±1.24 1.62	101.18±1.180 -1.52	28.11±0.89 1.04
% change	7 <sup>th</sup>	56.14±2.14 1.81	105.77±1.62 1.86	27.89±0.22 1.01
Radiation	1 <sup>st</sup>	82.38±1.54* 51.60	152.95±2.23* 48.87	38.28±0.38* 37.60
% change	7 <sup>th</sup>	81.66±2.39* 48.10	162.63±4.46* 56.62	39.02±0.63* 41.27
Clove oil + Radiation	ve oil + Radiation 1 <sup>st</sup>		105.02±3.89# 2.22	29.46±1.04# 5.90
% change	7 <sup>th</sup>	57.01±1.35#	108.87±3.34#	30.00±1.23#

Table (2)	Effect of cl	ove oil (150	) mg/kg b.wt	) on serun	n cholesterol,	triglycerides
and	Low density	y lipoprotei	n cholesterol	(LDL) in o	different anim	nal groups.

The % change of control.

Each value represents the mean of 6 observations ± S.E.

\* Significantly different from control values.

# Significantly different from the values of irradiated rats.

Table (3) showed a significant increase in FSH while a significant decrease in LH and testosterone hormone after exposure to irradiation, compared to control group. The supplementation of rats with clove oil for 21 days before irradiation induced significant decrease in FSH and a significant increase in LH and testosterone hormone, compared to their corresponding values in irradiated rats.

Groups	Days	FSH(mIU/ml)	LH(mIU/ml)	Testosterone (mg/ml)
Control	1 <sup>st</sup>	3.25±0.09	1.32±0.06	3.21±0.05
	7 <sup>th</sup>	3.28±0.09	1.36±0.06	3.18±0.05
Clove oil	1 st	3.50±0.13	1.38±0.03	3.48±0.12
	1	7.69	4.55	8.41
% ahanga	7 <sup>th</sup>	3.65±0.22	1.33±0.06	3.36±0.10
76 change	/	11.28	-2.21	5.66
De l'effer	1 st	6.24±0.40*	0.64±0.09*	1.64±0.14*
Kadiation	1	92	-51.52	-48.91
0/	<b>7</b> th	5.10±0.39*	0.57±0.09*	1.54±0.16*
% change	1	55.48	-58.09	-51.57
	1 <sup>st</sup>	3.89±0.39#	1.25±0.09#	3.22±0.18#
Clove on+Radiation		19.69	-5.30	0.31
0/ 1	7th	3.56±0.39#	1.24±0.11#	3.05±0.13#
% change	/	8.54	-8.82	-4.08

Table (3): Effect of clove oil (150 mg/kg b.wt.) on serum FSH, LH and testosterone hormones in irradiated rats.

The % change of control.

Each value represents the mean of 12 observations ± S.E.

\* Significantly different from control values.

# Significantly different from the values of irradiated rats.

#### DISCUSSION

A deleterious effect of radiation is the production of reactive oxygen species ROS, which result in damage to biomolecules (e.g. lipid, protein, amino acids and DNA) <sup>(38)</sup> and finally leads to many diseases. The over production of ROS results in oxidative stress: defined as the imbalance between pro-oxidation and antioxidants. As a result of this imbalance, a chain of lipid peroxidation is initiated; this induced cellular damage <sup>(39)</sup>. Antioxidant compounds may function as free radical scavengers, reducing agents and quenchers of singlet oxygen formation. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity <sup>(40)</sup>.

In the present study exposure of rats to gamma irradiation (6.5 Gy) resulted in marked significant elevation in lipid peroxides which was demonstrated by increased MDA level accompanied with depression in the antioxidant system (glutathione content superoxide dismutase and catalase enzymes). Zhang, *et al.* <sup>(41)</sup> and Chen, *et al.* <sup>(42)</sup> attributed the elevation in lipid peroxides contents to the peroxidation of the membrane unsaturated fatty acids

due to free radical propagation concomitant with inhibition of bio-oxidative activities.

Our results are in accordance with Marcillo, *et al.* <sup>(43)</sup> who reported that ionizing radiation induced excessive production of free radicals, enhances oxidative stress processes, which is associated with significant decrease in the oxidant status and accompanied by depleted antioxidant defensive system.

It was reported that free radicals formed by irradiation enhance lipid peroxidation and increase cytoplasmic membrane permeability to organic substances including enzymes <sup>(44)</sup>, data of our results are in accordance with Zahran, *et al.* <sup>(45)</sup> at 6.5Gy. GSH function to scavenge cellular oxygen radicals and help detoxification by conjugation with free radicals <sup>(46)</sup>. Therefore, the decline in GSH has often been considered to be indicative of increased oxidative stress and thus result in tissue damage <sup>(47)</sup>. The decrease in GSH could be due to inactivation of glucose 6-phosphate dehydrogenase, the main NADPH+H supplier essential for its activity <sup>(48)</sup>.

The decrease in SOD activity may result in an increased flux of super oxide in cellular compartments which may be the reason for the increased in MDA in our study. Clove has been reported to possess a potent antioxidant activity, which reduces the oxidative stress in the body <sup>(19)</sup>. Many investigators demonstrated the hepatoprotective properties of clove against chemical stress <sup>(10)</sup> and against physical stress <sup>(11)</sup>.

Administration of clove oil (150mg/kg b.wt.) to rats exposed to irradiation (6.5 Gy) result in significant amelioration in blood antioxidation status, these results are in agreement with Souza, *et al.* <sup>(49)</sup> and Ashu, *et al.* <sup>(50)</sup>, they stated that eugenol is a nontoxic, highly hepatoprotective agent, induce glutathione s-transferase and thereby it may facilitate the removal of toxic substance from the intestine, predominately due to its free radicals quenching ability <sup>(51-52)</sup> suggested that phenolic and allyl groups in eugenol structure and related components may inhibit lipid peroxidation at the level of initiation or at the level of propagation of free radical chain reaction like alpha tocopherol.Yadav and Bhatngar, <sup>(22)</sup> reported that clove oil has modulatory effect on iron–induced lipid peroxidation due to their polyphenolic contents, strong reducing power and superoxide radical scavenging activity, preventing the generation of ROS. Our results are also in agreement with Said, <sup>(53)</sup> who reported that eugenol decrease lipid and oxidative stress as well as improve intracellular antioxidant defense. Lee and Shibamato, <sup>(54)</sup> identified some aroma

chemicals such as eugenol, thymole and benzyl alcohol in the clove buds extract and examined them for their inhibitory effect on MDA formation. Clove aroma compounds exhibited the most potent antioxidant activities in suppression of lipid peroxidation.

Free radical impairs liver functions and can be a major reason of hormonal imbalance. This imbalance induces hyperlipidemia through its multiple effects on lipid metabolism, including increased synthesis of cholesterol and triglycerides<sup>(55)</sup>.

Cholesterol is derived from exogenous diet and endogenously from acetyl Co A in a series of biosynthesis reaction<sup>(56)</sup>, hypercholesterolemia is attributed to the increase of activation of HMG CoA reductase enzyme, the key regulatory enzyme in the reduction of the overall process of cholesterol synthesis<sup>(57)</sup>.

Results of the present study revealed increase in cholesterol, LDL and triglycerides serum levels in the irradiated rat on the 1<sup>st</sup> and 7<sup>th</sup> day post irradiation (6.5 Gy). This increase may be due to the stimulation of the liver enzymes responsible for the biosynthesis of fatty acids by gamma irradiation and to the mobilization of fat from adipose tissue to the blood stream leading to a hyperlipidemic state associated with elevation in LDL. The elevation could be attributed to an alteration of fatty acids in plasma<sup>(58)</sup>. The current results showed a significant decrease in serum total cholesterol, LDL and triglycerides in rats treated with clove oil, these results are in agreement with Teissedre, and Waterhouse, <sup>(59)</sup> and Ragab, <sup>(10)</sup>, they reported that eugenol inhibit in vitro oxidation of LDL + VLDL lipoprotion fractions isolated from rat plasma similar to the standard synthetic antioxidant butylated hydroxyloluene (BHT) and butylated hydroxyanisol (BHA). Clove oil might lower the plasma free fatty acid, TG and cholesterol levels, and simultaneously reduced the hepatic fatty acid oxidation. These changes were seemingly attributed to a suppression of the hepatic fatty acid synthase, glucose-6-phosphate dehydrogenase and also it may have been partly due to the decreased hepatic-3-hydroxyl-3-methyl glutaryl Coenzyme (HMG CoA) reducatse and acyl CoA: cholesterol acyl transferase (ACAT) activities as previously mentioned by Younies, <sup>(60)</sup>, who reported that clove oil improved the levels of cholesterol, TG and phospholipids in serum of hyperlipidemic rats.

Oxidative damage, elevated lipid peroxidation and the alteration of membrane properties can lead to germ cell death at different stages of

development and a decrease in the sperm count, the antioxidant therapy will act as a protective defense against oxidative stress and improve fertility parameters <sup>(61)</sup>. FSH aids in spermatogenesis or the production of sperms, FSH disorder may cause infertility and sexual dysfunctions. LH deficiencies cause sexual disinterest and low sperm count because of a lack of testosterone <sup>(62)</sup>.

In the current study, the significant increase in serum FSH in rats exposed to 6.5 Gy gamma irradiation post exposure might be attributed to disturbance inhibin of a peptide hormone produced by testicular tubules which acts by negative feedback mechanism to modulate the secretion production mechanism <sup>(63)</sup>. On the other hand, the present data showed that gamma radiation induced a significant decrease in LH 1 and 7 days post exposure of irradiation, this may be due to damage in Leyding cells where luteinizing hormone releasing hormone (LHRH) receptors are located <sup>(64)</sup>.

Testosterone is a steroid hormone from the androgen group. In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass and hair growth <sup>(65)</sup>. In addition, testosterone is essential for health <sup>(66)</sup>. In the present work, whole body gamma irradiation at a dose level of 6.5 Gy induced a decrease in the serum level of testosterone, the data comes in accordance with Varga, (67) who recorded decrease in plasma levels of testosterone after exposure to 3 Gy X-ray. Mc Taggart and Wills, <sup>(68)</sup>, said that gamma radiation to whole body, head or lower trunk region causes a fall in the circulating levels of testosterone and LH, they said that whole body radiation causes inhibition of the liver enzymes by a complex of interrelated effects the testis, anterior pituitary and possibly hypothalamus, the secretion of testosterone is under the control of hormones of the anterior pituitary and especially LH. In the present study, treated rats with clove oil pre-exposure to irradiation resulted in an improvement in the FSH, LH and testosterone hormones, these findings are supported by Tajuddin<sup>(69)</sup> who indicated that the 50% ethanolic extract of clove produced a significant and sustained increase in the sexual activity of normal male rats, without gastric ulceration and adverse effects. Thus the resultant aphrodisiac effectively of the extract lends support claims for its traditional usage in sexual disorders.

From our results, clove oil has the ability to stimulate the body self defense mechanisms against oxidative stress induced by radiation and increased antioxidant status by scavenging free radicals, inhibit lipid peroxidation which lead to improvement in the cell membranes resulted in hepatoprotective and genital protective properties in male albino rats.

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مجلة البحوث الإشعاعية والعلوم التطبيقية

مجلد ٤ عدد ٣ (ب) ص ص ٩٣٩ - ٥٩٥ (٢٠١١)

فاعلية زيت القرنفل كمضاد للأكسده ضد مخاطر الأشعاع فى ذكور الجرذان

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قسم بحوث بيولوجيا الإشعاع - المركز القومي لبحوث وتكنولوجيا الإشعاع هيئة الطاقة الذرية – مصر - القاهرة

تهدف الدراسة الى تقييم فاعليه تجريع زيت القرنفل وذلك لمده ٢١ يوم على التوالي بجرعه مقدار ها ١٥٠ ملي جرام/كجم من وزن الجرذ ولتحقيق ذلك تضمنت هذه الدراسة عدد (٤٨) من ذكور الجرذان البيضاء التي يتراوح وزنها من١٢٠-١٥٠جرام وقسمت إلى أربع مجموعات وتحتوى كل مجموعة على (١٢ جرذ): المجموعة الأولى/ جرذان المجموعة الضابطة. المجموعة الثانية/جرذان تم معالجتها عن طريق الفم بزيت نبات القرنفل (١٥٠ مللي جرام/كجم) لمدة ٢١ يوما متتالية ، المجموعة الثالثه جرذان تم تعرضها إلى جرعة مفردة من أشعة جاما (٦.٥ جراى) والمجموعة الرابعة/ جرذان تمت معالجتها بزيت نبات القرنفل (١٥٠ مللي جرام/كجم) لمدة ٢١ يوما ثمَّ تم تعرضها لأشعة جاما (٦.٥ جراي). وقد تم قياس كلا من محتوى الجلوتاثيون ، نشاط (انزيم السوبر أكسيد الديسموتيز، إنزيم الكتاليز)، المالوناداهيد في الدم، مستوي المحتوي الكلي للكلوستيرول ، الجلسيريدات الثلاثيه ، LDL وهرمونات LH ، FSH والتستستيرون. وتشير النتائج إلى أن الجرذان التي تعرضت للإشعاع (٦.٥ جراي) قد أظهرت ارتفاعاً ملحوظاً في مستوى المالوناداهيد يصاحبه نقص في مستوى مضادات الاكسده ،والمحتوي الكلي للكلوستيرول، والجلسيرات الثلاثيه وLDL و هرمونFSH، بينما لوحظ نقص هرمون التستسيبرون وهرمون LH علي اليوم الأول والسابع من التشعيع وتبعا لنتائج الدراسة فقد اظهر اعطاء زيت القرنفل (١٥٠ مللي جرام/كجم) قبل التعرض للإشعاع تحسن ذات دلاله احصائيه في القياسات السابق ذكرها. ولهذا تؤكد الدراسة أهميه زيت القرنفل كمركب طبيعي لتخفيف الإجهاد التأكسدي والتخلص من الشقائق الحرة الناتجة عن التعرض للإشعاع.