The efficacy of oral supplementation of GliSODin in reducing the Oxidative Stress in Rats Subjected to γ-radiation

N. Hanafi and S.Z. Mansour and S.F. Salama

Radiation Biology Dept., National Centre for Radiation Research and Technology (NCRRT), P.O.Box:29Nasr City, Egypt.

فاعلية التناول الفمى للجليسودين فى الإقلال من الضغط التأكسدى للجرذان المعرضة لأشعة جاما

نعمات حنفى أحمد و سـمية زكريا منصور و صفوت فريد سـلامة

خلاصـــــة

الغرض من هذة الدراسة هوتتبع مقدرة التناول الفمى للجليسودين (مركب خضرى يتركب من مستخلص بروتينى من القمح مضافا اليه السوبر أوكسيد ديزميوتيز المستخلص من الكونتالوب) فى الإقلال من الضغط التأكسدى للجرذان المعرضة لأشعة جاما. أستعملت فى تلك الدراسة جرذان معرضة لجرعة مقدارها ٦ جراى من أشعة جاما مع/أو التناول الفمى للجليسودين لمدة ٣ اسابيع (٥مج/١مل/جرذ) بعد التشعيع. فحصت بعض التغيرات فى مكونات الدم. كذلك عينت دلائل الضغط التأكسدى فى كل من البلازما و أنسجة كل من الكبد,الكلى والطحال.عينت كذلك التغيرات الهستوباثولوجية المحد ثة فى تلك الأنسجة المختبرة. أظفرت النتائج عن ذيادة معنوية فى مستوى دلائل اليبيدات الفوق مؤكسدة مع انخفاض معنوى فى مستوى مضادات الاكسدة نتيجة تعـرض الجرذان لأشعة جاما. وأوضحت الدرسات الهستوباثولوجية كثيرآ من التغيرات المهلكه. أظهرت النتائج بعد تناول الجرذان الفمى للجليسودين بمفردة أو بعد تعـرضها لأشعة جاما لمدة ثلاث اسابيع حدوث إنخفاض معنوي فى مستوى دلائل اليبيدات الفوق مؤكسدة مع زيادة معنوية فى مستوى مضادات الاكسدة كذلك أوضحت الدرسات الهستوباثولوجية حدوث نقص واضح فى التغيرات المهلكه.

وفقآ لهذه النتائج يمكننا الاستدلال على ان تناول الجليسودين كمكمل غذائى يقلل من الأضرار الناتجة عن التعرض للضغط التأكسدى .

Abstract

In a prospective study we tested the effect of oral supplementation of GliSODin (100% vegetable compound comprised of gliadin, a wheat protein extract bound to superoxide dismutase derived from cantaloupe) in reducing oxidative stress in rats subjected to γ-radiation. Adult male Swiss albino rats were used in this study, exposed to 6 Gy of γ-radiation and/or oral gavage with 5mg/ml/rat of GliSODin for 3 successive weeks. Oxidative stress biomarkers were evaluated in blood plasma, liver, kidney and spleen tissues. Some of the haematological parameters were investigated.

Histopathological observations in tissues were also detected. After γ-irradiation a significant decrease in haemoglobin (Hb)content,red blood cells RBCs count and haematocrite (Hct) level was recorded. Lipid peroxidation markers [malondialdehyde (MDA), lipid hydroperoxide (LHP) and conjugated diene (CD)] showed a significant increase. Histopathological examinations revealed a dangerous of alterations in liver, kidney and spleen tissues. However, GliSODin supplementation resulted in a significant decrease in lipid peroxidation either alone or after radiation exposure comparing to irradiated group. The antioxidant defence enzymes including superoxide dismutase (SOD), catalase (CAT) activities and reduced glutathione (GSH) content recorded a significant increase comparing to irradiated group. Histopathological examinations showed a melioration of radiation- induced damage. As a result, GliSODin could be considered a food supplement in the trials of minimizing oxidative stress disorders due to radiation exposure.

INTRODUCTION

Free radicals can be generated as by-products of the normal cellular redox processes or via the interaction of cells and tissues with a variety of external agents and processes (e.g. thermal or photochemical reactions, ionizing radiation or the action of xenobiotics) (Halliwell and Gutteridge, 1989). They documented that the effects of oxidative stress are the natural consequence of the oxygen metabolism. However, oxygen is absolutely necessary for the life processes, in particular cell respiration. These chemically unstable compounds carry free electrons that react with other molecules, in turn destabilizing them and thereby inducing a chain reaction. In particular, free radicals damage DNA, essential cellular proteins and membrane lipids (lipid peroxidation), which may lead to cell death (Menvielle-Bourg, 2005). In so-called "physiological conditions" there is a balance between the production of free radicals and antioxidant endogenous defence mechanisms. These mechanisms mainly involve specific enzymes (superoxide dismutase, catalase and glutathione peroxidase) as well as radical scavengers that trap free radicals such as vitamins (A, C, E), thiols and ß-carotene (Vouldoukis et al., 2004 a). However under certain conditions accompany the increased production of unstable oxygen derivatives: metabolism of sugars related to physical stress, lipid metabolism, immune response in particular toward microbial infections, exposure to radiation, pollution, smoking etc. Moreover, epidemiology studies indicate that the level of the antioxidant defences decrease with

age. When the antioxidant systems of defence are overloaded, oxidative stress (free radicals in excess) may occur. This may eventually contribute to the development of inflammatory or neurodegenerative diseases (Alzheimer's disease, Parkinson's disease), atherosclerosis, rheumatoid arthritis, Crohn's disease and even certain cancers. (Menvielle-Bourg, 2005). Free radicals are also known to contribute to the aging process. For this reason, we are currently witnessing the development of antioxidant products (functional food and drugs). However, their bioactivity with oral administration is often low, thereby limiting their efficacy. In addition, the products available on the market are made to correct a possible deficiency and do not specifically stimulate the antioxidant endogenous defences.

The powerful natural antioxidant enzyme superoxide dismutase (SOD) acts at the very source of the chain reaction resulting in reactive types of oxygen and therefore constitutes the first and one of the main links of the defence process against free radicals. Unfortunately, due to the fragility of its molecular structure, non-protected SOD is inactivated in the digestive tract. Thanks to a coupling process with gliadin, a protein extracted from wheat, a SOD of vegetable origin (melon extract rich in SOD) is now available orally. Several *in vivo* studies on animals as well as a clinical trial using healthy volunteers confirmed the preservation of the antioxidant activity of the SOD enzyme after oral administration; an action moreover combined with anti-inflammatory and immunomodulatory properties (Ioannis et al., 2004).

The determinant role of superoxide dismutase (SOD) in the antioxidant defence systems has been known since 1968. It is well known that superoxide ion (O_2–) is the starting point in the chain production of free radicals. At this early stage, superoxide dismutase inactivates the superoxide ion by transforming it into hydrogen peroxide (H_2O_2). The latter is then quickly catabolised by catalase and peroxidases into dioxygen (O_2) and water (H_2O). Different studies have confirmed that the production of H_2O_2 under the action of SOD is the triggering factor in the natural antioxidant defence mechanisms. SOD therefore seems to be the key enzyme in the natural defence against free radicals (Menvielle-Bourg, 2005).

Superoxide dismutases (SODs) are protein enzymes and their function specifically depends on their quaternary structure. All changes in the environment may, to a greater or lesser extent and more or less irreversibly, modify this structure and therefore the functionality of the SOD. In particular, during gastrointestinal passage, the quaternary structure is modified and the enzyme is inactivated. This is

why it is difficult to produce a SOD-rich food supplement that remains active when taken orally (Dugas, 2002). Therefore, to guarantee its efficacy, a SOD of exogenous origin has to be bioavailable, active in the body and protected during its digestive passage.

GliSODin is an original vegetable formula made from a SOD-rich melon extract (*Cucumis melo* LC), coupled with a Gliadin molecule, a protein extracted from wheat (GliSODin) (Coyler et al., 1987; Farre-Castany et al., 1995). Gliadin is a vegetable prolamine (biopolymer) that retains the active ingredient and delays its release in the small intestine. It is also bio-adhesive and in particular adheres to the wall of the small intestine. It progressively releases the SOD, counters its intestinal inactivation and eases its passage through the mucosa towards the blood circulation. Therefore, GliSODin is the first active SOD orally available.

The aim of the present study is to evaluate the efficacy of an oral supplementation of GliSODin in reducing the oxidative stress of rats' subjected γ-radiation

MATERIALS AND METHODS

Animals.

Adult male Swiss albino rats weighing 100-150 g obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccines, Cairo were used in this study. The animals were maintained on a commercial standard pellet diet and tap water *ad libitum* for 6 ~ 7 days before the experiment. All animal treatment procedures conformed to the national institutes of health (NIH) guide lines (NHI, 1985).

Radiation facility:

Whole body gamma irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using Caesium -137 in a Gamma cell-40 Irradiator (Atomic Energy of Canada Limited, Canada). Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.61 Gy min^{-1}.

GliSODin

GliSODin is the First Bio-active SOD Available orally. GliSODin an original vegetable formula made from a SOD-rich melon extract (*Cucumis melo* LC), coupled

with a Gliadin molecule, a protein extracted from wheat. This compound was purchased from Novus Research, Inc. Gilbert, Arizona 85234. ORDERS: 800-244-2438. The experimental animals were orally supplemented by 5mg/ml/rat GliSODin for 3 weeks.

Experimental design

Rats were randomly divided into four groups, each consisting of 6 animals. **G1:** served as control rats. **G2:** 6 rats orally supplemented with GliSODin for 3 weeks. **G3:** 6 rats exposed to 6 Gy whole body γ- irradiation **G4:** 6 rats exposed to 6 Gy of whole body γ -radiation followed by an oral supplementation with GliSODin, two hours after radiation exposure for 3 weeks.

Biochemical analysis:

24 hours after the last treatment all rats were sacrificed under mild anaesthesia. Blood samples were collected from the heart in two portions: one portion was collected in heparinized tubes centrifuged at 3000 r. p. m. for 15 minutes to separate plasma for biochemical studies. The remaining portion was used for hematological studies. Liver, kidney and spleen were excised immediately and were homogenized in ice cold phosphate buffer (0.1M/pH 7.4) to give 10% homogenates for determination of malondialdehyde (MDA), lipid hydrperoxide (LHP), lipid conjugated dienes (CD), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) assays. The MDA content (a measure of lipid peroxidation), was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the method of Yoshioka et al., (1979). LHP were evaluated according to the method of Jiang et al., (1992). However, CD were assayed according to the method of Rechnagel and Gglende,(1984). GSH in the different tissues was assayed by the method of Jollow et al. (1974). SOD activity in different homogenates was determined according to the method of Minami and Yoshikawa (1979). CAT activity was assayed by the method of Claiborne (1985). All reagents were purchased from Sigma (Sigma-Aldrich Corp, St. Louis, MO, USA).

Haematological studies

RBCs count were determinated using improved Neubauer chamber according to Dacie and Lewis (1991). Hb content were evaluated according to the procedure described by was determined colourimetrically as cyanmethaemoglobin in grams per decilitre using Spectrum Diagnostic Kit according to Teitz (1990). The ratio of

erythrocytes to plasma in percent (Hct) was measured as the volume of erythrocytes per 100 ml blood after Seivered (1964).

Histopathological study

The samples were fixed in 10% neutral buffered formalin, dehydrated through alcohols, cleared in xylene and then embedded in paraffin wax. Sections (5 mm thick) were stained with haematoxylin and eosin (Drury and Wallington, 1976).

Statistical analysis

The SPSS/PC computer program (version 10) was used for the statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA) followed by Post hoc test (LSD alpha) for multiple comparisons. The data were expressed as mean ± standard error (SE). P values< 0.05 were considered to be statistically significant.

RESULTS

1-Biochemical analyses:

1-1-Effect of GliSODin on some haematological parameters:

The protective capacity of GliSODin on some haematological parameters is shown in table (1). γ- Radiation exposure leads to a significant decrease in Hb, RBCs and (Hct) levels compared to control values. By direct gavages of experimental animals with GliSODin for 3 weeks, either alone or post irradiation exposure haematological parameters revealed a significant amelioration in comparison with those of irradiated group.

Table (1): ***Effect of GliSODin on some haematological parameters.***

Parameters	G1	G2	G3	G4
Hb (mg/dl)	$12.94 + 0.26^{bcd}$	$15.39 + 0.48^{acd}$	$7.03 + 0.54^{abd}$	$8.78 + 0.37^{abc}$
RBCs($10^6/m^3$)	$5.39 + 0.11^{bcd}$	$6.41 + 0.20^{acd}$	$2.93 + 0.22^{abd}$	$3.66 + 0.15^{abc}$
Hct(%)	$40.10 + 1.91^{bcd}$	$44.81 + 1.81^{acd}$	$25.94 + 1.57^{abd}$	$30.00 + 2.41^{abc}$

Each value represents the mean of 6 records ± S.E.
Means with different superscripts are significantly different at the 0.05 level
G1: *control group.* ***G2:*** *GliSODin supplementation group.*
G3: *whole body γ- irradiation group.* ***G4:*** *γ- irradiation and GliSODin group.*

1-2-Effect of GliSODin supplementation on γ- irradiation induced lipid peroxidation in rats:

To verify any changes in lipid peroxidation subsequent to exposure to γ-radiation, the concentration of LHP, CD and MDA in plasma, liver, kidney and spleen tissues of experimental animals was measured. Exposure of rats to γ-radiation showed an increase in LHP, CD and MDA levels in plasma as well as in liver, kidney and

spleen tissues compared to G1 control group. A significant amelioration effect was detected in lipid peroxidation levels, when the experimental animals received GliSODin either alone or after exposure to 6Gy γ- radiation, (table 2).

Table (2): Effect of oral administration of GliSODin on lipid peroxidation levels in rat tissues.

Organs	Parameters	G1	G2	G3	G4
Plasma	LHP (µg/ml)	60.57 ±1.85	61.04 ± 2.28	66.97 ± 2.91	63.77 ± 2.09
	CD	7.80 ± 0.16^{c}	8.00 ± 0.06^{c}	8.73 ± 0.09^{abd}	8.10 ± 0.06^{c}
	MDA (µg/ml)	79.91 ± 1.89^{c}	74.16 ± 1.88^{c}	89.24 ± 1.87^{ab}	85.66 ± 1.34^{ab}
Liver	LHP (µM /ml)	86.16 ± 5.99	86.016± 0.87	93.71 ± 2.25	88.47 ± 2.26
	CD	2.20 ± 0.01^{cd}	2.37± 0.02^{c}	2.73 ± 0.12^{abd}	2.43 ± 0.01^{ac}
	MDA (µg/g tissue)	96.99 ± 1.04^{cd}	97.48 ± 1.37^{cd}	119.98 ± 1.2^{abd}	106.48 ±1.55^{abc}
Kidney	LHP (µM / ml)	88.58 ± 2.02 ^{cd}	89.38 ±0.80^{c}	98.58 ± 3.17^{ab}	95.22 ± 1.50^{a}
	CD	2.08 ± 0.06^{cd}	2.11± 0.07^{cd}	2.75 ± 0.05^{abd}	2.46 ± 0.04^{abc}
	MDA (µg/g tissue)	80.81 + 1.10^{cd}	79.65 + 1.04^{cd}	92.81 + 1.10^{abd}	87.90 + 1.09^{abc}
Spleen	LHP (µM / ml)	83.77 ± 1.95 ^{cd}	84.16 ± 1.63^{cd}	96.31 ± 2.32^{ab}	91.34± 0.82^{ab}
	CD	1.77 ± 0.07^{bcd}	2.01 ± 0.07^{ac}	2.82 ± 0.08^{abd}	2.15 ± 0.05^{ac}
	MDA (µg/g tissue)	77.00 + 2.11^{cd}	78.82 + 1.71^{cd}	97.15 + 2.56^{abd}	86.07 + 0.68^{abc}

Ligands as in table 1 .

1-3-Effect of GliSODin supplementation on γ- irradiation induced decrease in activity of antioxidant enzymes:

Table 3 presentes the effect of gastric intubation of GliSODin on the antioxidant enzymes of whole body irradiated rat tissues. Exposure of animals to γ-radiation showed a significant decrease in GSH level either in plasma, liver, kidney or spleen tissues compared to control group. Non significant change in GSH levels was detected in the tested tissues of the experimental animals administrated GliSODin alone for 3 weeks. However, GliSODin supplementation showed a pronounced ameliorative effect on GSH level in plasma, liver, kidney and spleen tissues of the 6 Gy whole body γ- radiated animals. The same trend was detected in SOD level in the different experimental groups and in the different tissue organs. Also, the Exposure of the experimental animals to 6 Gy γ- radiation revealed a significant decrease in CAT level either in plasma, liver, kidney or spleen tissues compared to control group. However, the supplementation of rats with 5mg/ml/rat GliSODin for 3 weeks in G2 or

G4 groups induced a significant improvement effect on CAT values in comparison with G3 irradiated group.

Table (3): Effect of GliSODin supplementation to rats on the antioxidant enzymes (GSH, SOD and CAT) levels in irradiated rat tissues:

Organs	Parameters	G1	G2	G3	G4
Plasma	**GSH** (mg/dl)	45.99 ± 3.59^{c}	49.42 ± 2.06^{c}	34.46 ± 2.36^{abd}	47.59 ± 1.45^{c}
	SOD (u/ml)	7.53± 0.92^{c}	7.58± 0.24^{c}	6.20± 0.16^{abd}	7.19± 0.13^{c}
	CAT (µmol/ ml)	203.22 ±2.38^{c}	215.89±12.31^{c}	120.45 ± 4.93^{abd}	196.33 ±4.45^{c}
Liver	**GSH** (µg/g tissue)	26.39 ± 0.45^{c}	26.86 ± 0.42^{c}	22.24 ± 0.77^{abd}	25.53 ± 0.30^{c}
	SOD (u/g tissue)	12.40± 0.16^{cd}	12.50 ± 0.22^{cd}	9.21 ± 0.16^{abd}	11.80±0.13^{abc}
	CAT (µmol/g tissue)	161.48±3.78^{bc}	201.11±8.56^{acd}	110.80±10.69^{abd}	160.73±2.69^{bc}
Kidney	**GSH** (µg/g tissue)	23.10 ± 0.32^{cd}	23.52 ± 0.33^{cd}	18.92 ± 0.42^{abd}	20.70±0.30^{abc}
	SOD (u/g tissue)	8.01 ± 0.06^{c}	8.26± 0.18^{c}	6.79 ± 0.36^{abd}	7.81 ± 0.28^{c}
	CAT (µmol/ g tissue)	142.89± 6.29^{c}	133.22 ± 5.63^{c}	108.56 ±3.66^{abd}	131.86 ±2.60^{c}
Spleen	**GSH** (µg/g tissue)	21.95 ±0.57^{cd}	22.21 ± 0.95^{cd}	17.43 ± 0.32^{abd}	19.59±0.49^{abc}
	SOD (u/g tissue)	7.03 ± 0.04^{c}	7.13 ± 0.16^{c}	5.74 ± 0.17^{abd}	6.95 ± 0.11^{c}
	CAT (µmol/ g tissue)	145.48 ±4.92^{c}	140.89 ± 3.51^{c}	122.11 ±2.28^{abd}	143.89 ±3.26^{c}

Ligands as in table 1

2-Histopathological observations

2-1- liver:

Fig. (1) presentes a section derived from control rat liver which composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Some cells have two nuclei each.

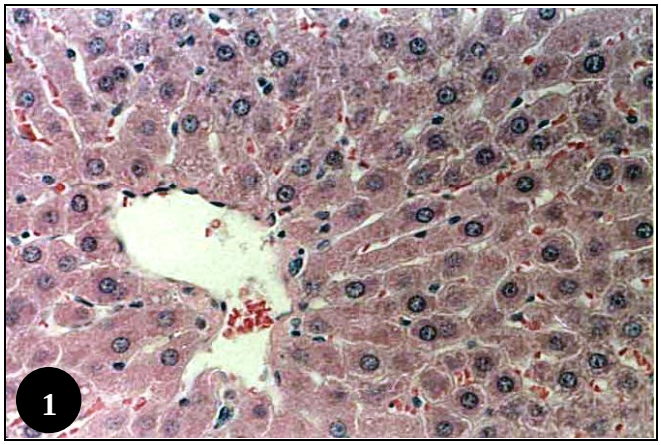

Fig. (1): Photograph of a section in liver of control rat. H&E, x400

Exposure of rats to 6 Gy whole body γ- radiation desplaied hepatocellular vacuolization (↑) and sinusoidal congestion in addition to the presence of pecnotic nuclei (fig. 2). Also dilatation of the central vein (★) was distinctly observed in fig. (2a). GliSODin supplementation of rats for 3 weeks recorded mild alterations in hepatic morphology, including the presence of fatty hepatic infiltrates and cytoplasmic vacuolization (fig. 3). Retrain to normal observation in liver tissue section when the irradiated group orally administrated with GliSODin for a period of 3 weeks (fig.4).

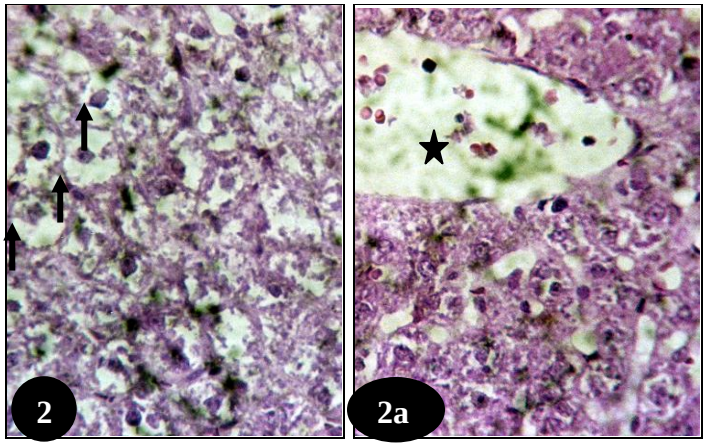

Fig. (2 and 2a): Photograph of sections in liver of irradiated rats. H&E, x400

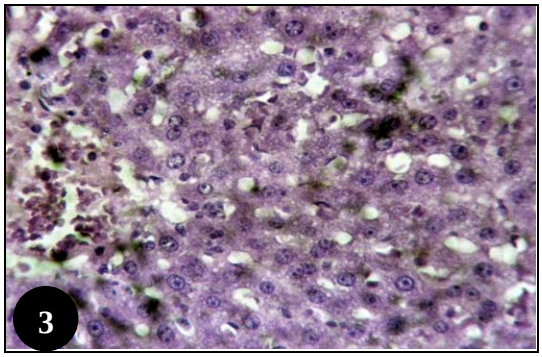

Fig. (3): Photograph of section in liver of rat treated with GliSODin H&E, x400

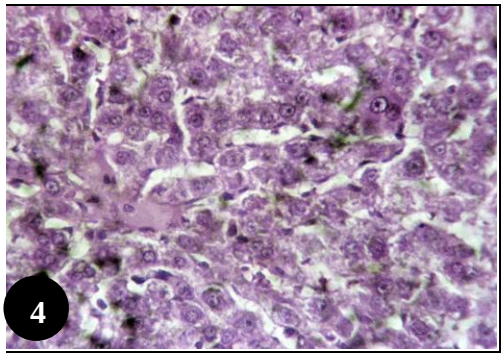

Fig. (4): Photograph of sections in liver of irradiated rat treated with GliSODin. H&E, x400

2-2- Kidney:

Fig. (5) shows the normal histological structure of the inner medulla of control rat kidney. It is composed of a huge number of functional filtering units, nephrone. Each nephrone consists of a dilated portion, the renal corpuscle; the proximal convoluted tubule; the thin and thick limbs of the loop of Henle; and the distal convoluted tubule. The renal corpuscle consists of a tuft of capillaries, the glomerulus, surrounded by a double walled epithelial capsule called Bowman's capsule (↑). Between the two layers of the capsule is the urinary space, the proximal convoluted tubule and distal convoluted are observed.

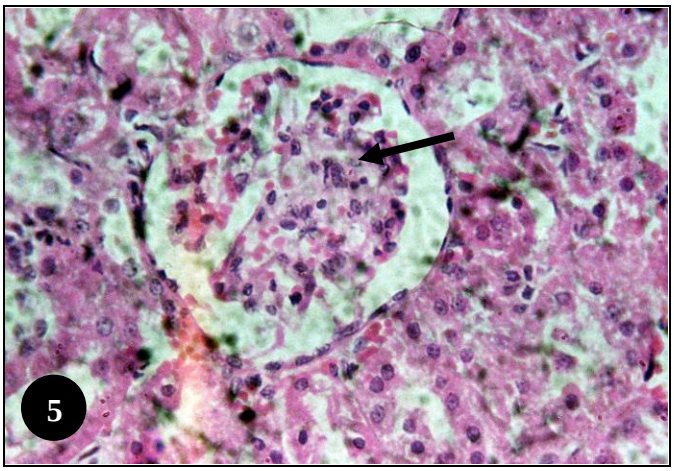

Fig. (5): Photograph of a section in kidney of control rat. H&E, x400

Histological examination of kidneys of animals after exposure to 6 Gy of γ-radiation, showed tubular dilatation with atrophy and some kidney tubules were vacuolated with marked tubular damages in comparison to the control tissue section(↓) (Fig. 6a). Also, some glomeruli were atrophied and congested (curved arrow) (fig.6).

In fig. (7) by gavages of the experimental animals by GliSODin for 3 weeks showed some alterations in the kidney tissue section evantually by enlargement of vascular glomeruli and tightly filling the Bowmann's capsule (↓). Retrain to normal observations in tubular and glomeruli (↓) parts of kidney tissue sections were recorded when the irradiated group received GliSODin for 3 weeks (fig. 8).

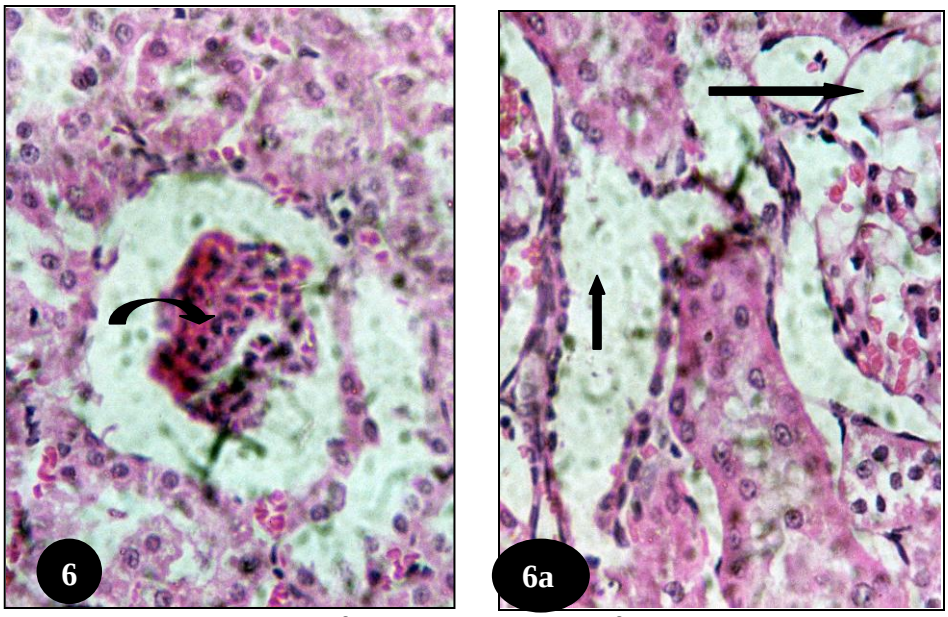

Fig. (6& 6a): Photograph of sections in kidney of irradiated rat. H&E, x400

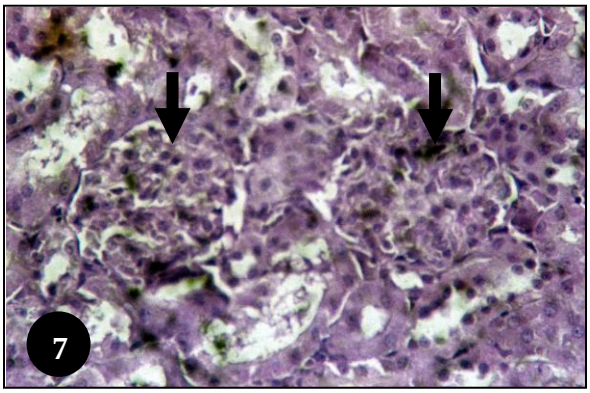

Fig. (7): Photograph of a section in kidney of control rat treated with GliSODin.

H&E, x400

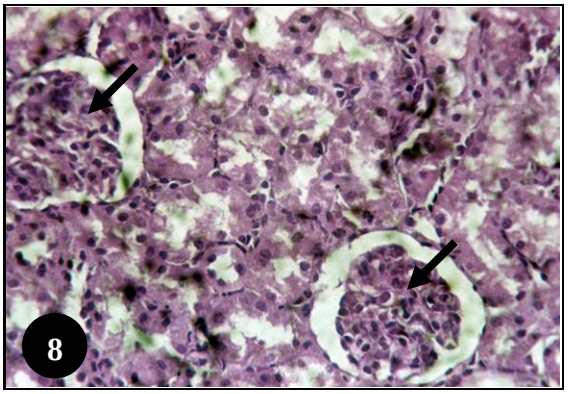

Fig. (8): Photograph of a section in kidney of irradiated rat treated with GliSODin.

H&E, x400

2-3- spleen:

Fig. (9) represents the normal histological structure of the spleen. It comprises of 2 functionally and morphologically distinct compartments, the red pulp (R) and the white pulp (W).

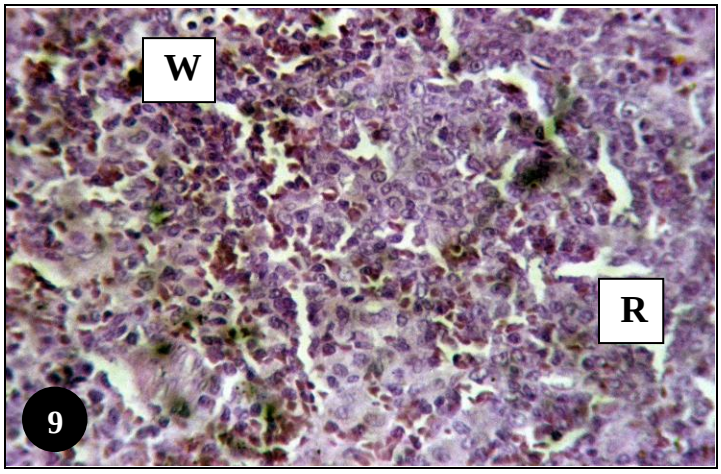

Fig. (9): Photograph of a section in spleen of control rat. H&E, x400

Spleen tissue sections of rats exposed to 6 Gy of γ–radiation revealed enlarged white pulp with increased sinusoidal spaces, when compared to the control group. Some white pulp appeared to be fused. This disorganization is due to hyperplasia of the lymphoid tissue. Some apoptotic spleenocytes were also detected. Normal observations in spleen tissue sections when the experimental animals orally administrated by GliSODin for 3 weeks either alone or combined with γ– irradiation group.

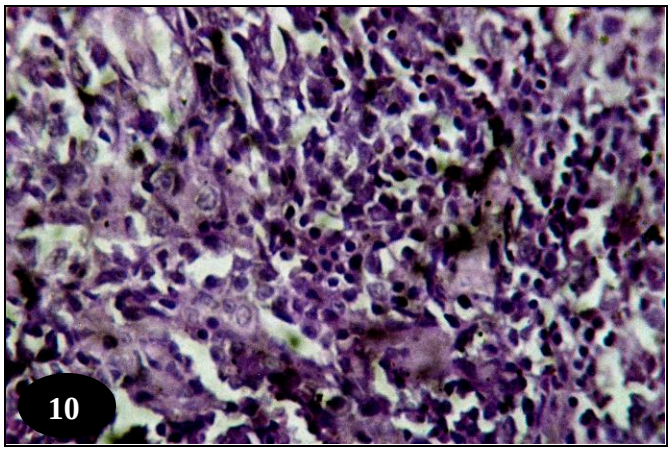

Fig. (10): Photograph of a section in spleen of irradiated rats. H&E, x400

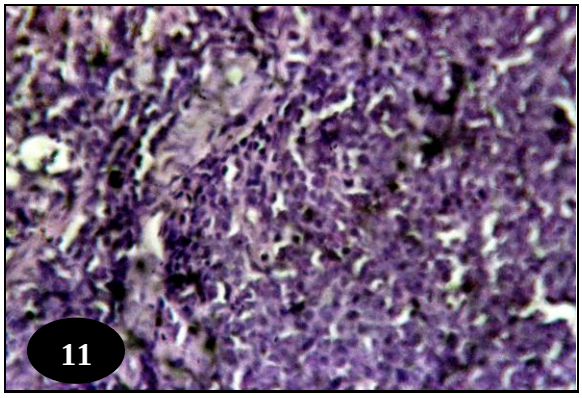

Fig. (11): Photograph of a section in spleen of control rat treated with GliSODin.
H&E, x400

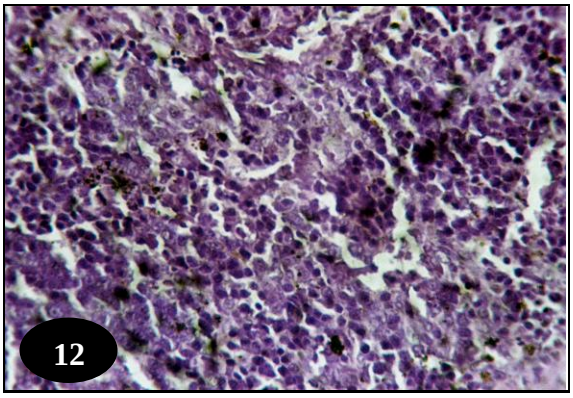

Fig. (12): Photograph of section in spleen of irradiated rats treated with GliSODin.
H&E, x400

DISCUSSION

Radiation is known to induce oxidative stress through generation of ROS, resulting in imbalance of pro-oxidants and antioxidants in the cells, which is suggested to culminate in cell death (Aoshima et al., 1997; Lin et al., 1995). Radiation produces disruption of sensitive molecules in the cells including nucleic acids, proteins and lipids, whereas the other actions occur when radiation interacts with water molecules in the cell, resulting in the production of highly reactive free radicals, such as $^{\cdot}OH$, $^{\cdot}H$, and e^{-}_{aq}. The free radicals can change the chemicals in the body (Shirazi et al., 2007) and can induce the cellular antioxidant defence enzymes such as SOD, glutathione peroxidase and CAT (Zhang et al., 2005). The involvement of free radical scavengers in protecting against radiation exposure damage was highlighted when scientists found that whole body radiation exposure decreased the total antioxidant capacity of organisms and that the levels of known antioxidants such as ascorbic acid and uric acid were depleted.

Radiation induced concomitant clinical problems of a tendency toward uncontrolled haemorrhage, decreased resistance to infection, and anaemia (Nunia and Goyal 2004). Our studies revealed that the exposure to γ- radiation exposure leads to a significant decrease in certain haematological parameters(RBCs ,Hb content and Hct value) . By oral gavages of experimental animals with GliSODin for 3 weeks, SOD level significantly ameliorated in comparison with that irradiated group. Claus et al., (2004) reported that the orally effective SOD–wheat gliadin mixture attenuated

oxidative DNA stress when the experimental animals exposed to hyperbaric oxygen related to oxidative stress.

In the present study studies the non significant change in GSH, CAT and SOD levels in plasma when the experimental animals received GliSODin for 3 weeks are consistent with the findings of Claus et al., (2004) who did not find a significant changes in blood SOD and CAT activities after oral SOD ingestion. However, in another study increased SOD activity was detectable both in erythrocytes and plasma, when GliSODin was administered in a higher dose and over a longer period of time (Postaire et al., 2000; Vouldoukis et al., 2004 b). Also, the ameliorative effect which was detected when GliSODin was supplemented for 3 weeks after γ-radiation exposure could be related to its role in reinforcing the endogenous anti-oxidant defence (Claus et al., 2004). Or may be the oral ingestion of exogenous SOD extracts strengthened the antioxidant capacity inside the body (Maki et al., 2007).

Previous studies showed that γ- radiation damages cell membrane by altering lipid profile and LPO. Lipid peroxidation begins with the formation of a lipid free radical, which rearranges to form a diene. Partial oxidation results in the formation of a lipid peroxy radical which takes up a hydrogenation to form lipid hydroperoxide or lipid endoperoxide. Malondialdehyde is a breakdown product of unsaturated fatty acids (Sushama and venugopal, 1988). Sung (2003) reported that oxidative damage is the mismatched redox equilibrium between the production of reactive oxygen species (ROS) and the ability of the cell to defend against them. Oxidative damage, therefore, occur when the production of ROS increases and scavenging of ROS decreases. Ionizing radiation such as γ-rays is thought to produce free radicals in the cell. These can cause a number of diseases, and are involved in the detrimental effect of ionizing radiation.

In the present study when the experimental animals exposed to 6 Gy γ-radiation showed increase in LHP, CD and MDA levels, decrease in GSH, SOD and CAT levels in liver, kidney and spleen tissues. The increase in LHP, CD and MDA levels in agreement with Sussan et al., (2002) who demonstrated that free radical attack on hydrophilic moiety, along with lipid peroxidation, which may constitute the principal mechanism of radiation induced damage of biological membranes (Edimecheva et al., 1997). The depletion in GSH content may be due to oxidation of sulfhydryl group or due to the diminished activity of glutathione reductase (GR),

(Sarkar et al., 1998). Erden and Kahraman (2000) recorded decline in GR in liver, kidney and spleen tissues of rats exposed to irradiation and contributed this decline to the inactivation of SH groups existing at the active site of the enzyme molecule by $^{\cdot}OH$ and $^{\cdot}O_2$ which are formed as result of irradiation. Additionally Irshad and Chaudhuri (2002) indicated that there is a close relationship between depletion of GSH and antioxidant enzymes and increase in lipid peroxidation based on the excessive formation of ROS as well as the depletion of cellular antioxidants. Also, ROS formation affects the antioxidative enzyme (SOD) which catalyzes the dismutation of superoxide radical anion (O2.-) into less noxious hydrogen peroxide (H_2O_2), that is further inactivated by degradation by glutathione peroxidase (GPx) and CAT. The reduction of H_2O_2 into water by GPx is accompanied by the conversion of glutathione from reduced form (GSH) into oxidized form (GSSG) (Kwiecieñ *et al.*, 2002).

GliSODin effectiveness is due to the two unique compounds from which its name is derived, Gliadin and SOD. GliSODin is protected by gliadin, a wheat protein that guards SOD during digestion, making GliSODin a completely vegetarian product. Gliadin has bio-adhesive properties that make GliSODin "stick" to the epithelial cells in the small intestine, presenting the SOD for utilization by the body. In the present study GliSODin supplementation for 3 weeks recorded non significant change in GSH, SOD and CAT levels in liver, kidney and spleen tissues.

The amelioration effect in GSH, SOD and CAT levels in tissue when the irradiated group orally administrated by GliSODin for 3 weeks may be attributed to the presence of SOD (Abou-Seif et al., 2003).

The Microscopic examination of liver tissue of irradiated rat revealed hepatocellular vacuolization, sinusoidal congestion in addition to the presence of dilatation of the central vein and picnotic nuclei associated with the significant increase in lipid peroxidation and the significant decrease in GSH level which are consistent with the results of Hanafy et al., (2007).

GliSODin consumption recorded a mild alteration in hepatic morphology, including the presence of fatty hepatic infiltrates and cytoplasmic vacuolization. When large amounts of antioxidants nutrients are taken, they can also act as pro-oxidants by inducing oxidantive stress (Podmore et al., 1998; Palozza, 1998). Further more pro-oxidant activity can induce either beneficial or harmful effects in biologic system (Palozza, 1998). Also Hanafi et al., (2007) found that under normal condition,

excess of antioxidants in blood produces toxic radicals which can be uptaken by various tissue recycling mechanisms, and oxidized-low density lipoprotein (LDL) particles that may be formed are rapidly cleared by the liver. In contrast, the same antioxidants-derived radicals accumulate within the vessel wall and may not be as readily cleared, resulting in a prooxidative effect (Steinberg et al., 1989).

Administration of GliSODin for 3 weeks post irradiation showed significant amelioration of the radiation induced damage that may resulted from delayed depolarization response and an increase in the resistance to apoptosis induced by oxidative stress (Joanny , 2005).

In Kidney tissue section the exposure to 6 Gy of γ-radiation, showed tubular dilatation with atrophy and some kidney tubules were vacuolated with marked tubular damages. Owoeye et al., (2008) hypothesized that the pathophysiology of radiation nephritis is due to cellular injury caused by ionizing radiation. Also, all components of the kidneys are affected, including glomeruli, mesangium, blood vessels, tubular epithelium, and interstitium. Cohen (2007) explained that renal injury caused by ionizing radiation is initiated by oxidative injury to deoxyribonucleic acid (DNA), that it is a genotoxic injury. It is established that tissue injury elicits acute inflammation whose features among others include swelling of the affected part. This is due to accumulation of exudates particularly fluid, proteins, and cells from local vessels unto the damaged part (Stevens and Lowe, 2000). The same alterations represented in kidney tissue section may be that some antioxidants-derived radicals accumulate within the vessel wall and may not be as readily cleared, resulting in a prooxidative effect (Steinberg et al., 1989). Retrain to normal observations in tubular and glomeruli parts of kidney tissue sections mentioned the antioxidant effect of GliSODin in promoted cellular antioxidant status and protected against oxidative stress-induced cell death (Thomas. 2005).

In G3 Spleen tissue sections revealed enlarged white pulp with increased sinusoidal spaces. Some white pulp of G3 tissue sections appeared to be fused. Also some of apoptotic spleenocyts were detected. This disorganization was due to hyperplasia of the lymphoid tissue (Al-Glaib et al., 2008). The normal observations in spleen tissue sections when the experimental animals orally administrated by GliSODin for 3 weeks either alone or after γ– irradiation group represented its important role in protecting from oxidative damage (Vouldoukis et al., 2004 b).

In conclusion, Glisodin is a unique supplement, especially appropriate in the fight against free radicals overloading, in particular when the body's own natural defences are weakened. In exposure to γ-radiation it prevents certain chronic disorders involving oxidative stress and or slows down their evolution, thereby reducing the hazardous effect of exposure to ionizing radiation.

REFERENCES

Abou-Seif, M.A. M., El-Naggar, M.M., EL-Far, M., Ramadan, M., Salah, N., Clinica chimica, 337(1-2), pp. 23-33 (2003).

Al-Glaib, B., Al-Dardfi, M., Al-Tuhami, A., Elgenaidi, A., Dkhil, M., Libyan J Med, AOP: 080107(2008).

Aoshima, H., Satoh, T. and Sakai, N., Biochim. Biophys. Acta., 1345: 35–42, (1997).

Brites, F. D., Evelson, P. A., Christiansen, M. G., Nicol, M. F., Basilico, M. J., Wikinski, R. W. and Llesuy, S. F., Clin. Sci. (Lond).,96,381-385,(1999).

Cazzola, R., Russo-Volpe, S., Cervato, G. and Cestaro, B., Eur. J. Clin. Invest., 33,924-930 (2003).

Claiborne, A.: Catalase activity. In: Greenwald, R.A. (Ed.), CRC Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, FL, pp. 283–284,(1985).

Claus, M. M., Yvonne, G., Michael, K., Peter, R., GU¨ Nter, S. and Xavier, L., Free Radical Research, 38(9), pp. 927–932, (2004).

Cohen, A. H., Fundamentals of Renal Pathology. Agnes B. Fogo, Cohen, A. H., J., Charles J., Jan A. B. and Robert B. C.3-17 (2007).

Coyler, J., Kumar, P.J., Waldron, N.M., Clark, M.L. and Farthing, M.J., Clinical Sciences, 72, 593–598, (1987).

Dacie, S. T. and Lewis, S. M., Practical haematology.7th ed. Churchill, Levingston. Medical Division of Longman Group. UK. Ltd., (1991).

Drury RA., Wallington EA., Carlton's histological techniques, 6th ed., London: Oxford University Press; p. 139–142,(1976).

Dugas, B.,Free Radic. Biol. Med., 33, S64,(2002).

Edimecheva, I.P., Kisel, M.A., Shadyro, O.I., Vlasov, A.P. and Yurkova, I.L., Int. J. Radiat. Biol. 71 (5): 555-560, (1997).

Erden Inal, M. and Kahraman A. Toxicology, 23,154(1-3):21-9, (2000).

Evelson, P., Gambino, G., Travacio, M., Jaita, G., Ve rona,J., Maroncelli, C., Wikinski, R., Llesuy, S. and Brites, F., Eur. J. Clin. Invest 32, 818–825, (2002).

Farre-Castany, M.A., Kocna, P. and Tlaskalova-Hogenova, H.,Folia Microbiologica, 40, 431–435, (1995).

Halliwell, B. and Gutteridge, J.M.C., 2nd ed., Clarendon Press, Oxford, pp. 188-207,(1989).

Hanafi, N., Abd Baset, El A., Mohamed, S. G., Amany, A T. and Manal, S.E. ,The Egyptian Journal of Hospital Medicine.26, 22-30, (2007).

Hanafy, N., Hussien, A.H. and Heba ,H. M., Isotope & Rad. Res. 38(3),751-764, (2007).

Hong, Y., Hong, S., Zhang, Y. H. and Cho, S. H., American Association Clinical Chemistry, Annual Meeting, Los Angeles, U.S.A., p.A5 8, (2004).

Ioannis, V., Dominique, L., Caroline, K., Philippe, C., Alphonse, C., Dominique, M., Marc, C. and Bernard, D., Journal of Ethnopharmacology 94, 67–75, (2004).

Irshad, M. and Chaudhuri,PS. Indian J Exp Biol. 40(11):1233-9, (2002).

Jiang, ZK., Hunt, JV. and Wolf, SP.,Anal Biochem, 202, 384 -389, (1992).

Joanny Menvielle ,B. F., Phytothérapie, 3,1-4, (2005).

Jollow, D., Mitchell, L., Zampaglione, N. and Gillete, J., Pharmacology, 11,151–169, (1974).

Kwiecieñ, S., Brzozowski T., Konturek SJ., J Physiol Pharmacol, 53, 39-50, (2002).

Lin, KT., Xue, JY., Nomen, M, Spur, B. and Wong, PY., J. Biol. Chem.; 270, 16487–90, (1995).

Maki, K., Hayami, N., Shinsuke, H., Akiharu, W., and Kunihisa, S., Journ al of Health Science, 53(5) 608–614,(2007).

Menvielle-Bourg F. J., Doctor in Pharmaceutical Sciences, Fabienne Joanny, Consulting and Licensing, Paris, France in Phytothérapie, 3 (2005).

Minami, M. and Yoshikawa, H., Clin. Chim. Acta; 92:337–342, (1979).

NIH "National Institutes of Health", National Institutes of Health, Bethesda, MD., in Publication ,85(23), (1985).
Nunia, V. and Goyal, P. K., J. Radiat. Res., 45, 11–17, (2004).

Ohkawa, H., Ohishi, N. and Yagi K., Anal. Biochem., 95(2),351–358,(1979).

Owoeye, O., Malomo, A. O., Elumelu, T. N., Salami, A. A., Osuagwu, F. C., Akinlolu, A. A., Adenipekun, A. and Shokunbi, M. T. ,Int. J. Morphol., 26(1),69-74, (2008).

Ozbay, B. and Dulger, H., J. Exp. Med. Tohoku,197,119-124, (2002).

Palozza, P., Nutr, Rev., 56, 257-265,(1998).

Podmore, ID., Griffiths, HR., Herbert, KE., Mistry, N., Mistry, P. and Lunec, J., Nature, 392,559, (1998).

Postaire, E., Regnault, C., Rock-Arveiller, M., Stella, V., Brack, M. and Sauzie`res, J., United States Patent, 6045809, (2000).

Rechnagel, RO. and Gglende, EA., 105, 331-337,(1984).

Sarkar, S., Yadav, P. and Bhatnagar, D.J. Biometals., 11: 153-157, (1998).

Seivered, C., Haematology for medical technologists. Eds. Lea and Febiger, Pheladelphia, pp.34, (1964).

Shirazi, A., Ghobadi, G. and Ghazi-Khansari, G. A., J. Radiat. Res., 48, 263-72,(2007).

Steinberg D., Parthasarathy S., Carew, TE., Khoo, JC. and Witztum, JL, N. Engl. J. Med., 320,915–924, (1989).

Stevens, A. and Lowe, J. S. Human Histology. 2nd ed. London, Mosby,. pp 78, 358, (1999).

Sung, J. S., Journal of Biochemistry and Molecular Biology, 36(2), pp. 190-195, (2003).

Sushama, K. S. and Venugopal, P. M., Biosci, J., 139 (3), pp. 257–262, (1988).

Sussan, K. A., Mehrali, M. J., Efaht, S. and Amina, K., Iranian Biomedical Journal 6 (1), 37-41, (2002).

Teitz N., Clinical Guide to Laboratory Tests.2rd ed. Philadelphia.WB Saunders. p. 566, (1990)

Thomas, PL., Business Services Industry.Phytotherapy Research, (2005).

Vouldoukis, I., Conti M., Kolb J.P., Calenda A., Mazier D. and Dugas B., Curr. Trends Immunol., in press, (2004) (a).

Vouldoukis, I., Conti, M., Krauss, P., Kamaté, C., Blazquez, S., Tefit, M., Mazier, D., Calenda, A. and Dugas, B., Phytother. Res., 18 (12), 957-62, (2004) (b).

Yoshioka, T., Kamada, K. Shimadam T. and Mori, M., Am. J. Obstet. Gync., 135, 372. (1979).
Zhang, B., Su Y., Ai G., Wang Y., Wang T., Wang F. J. Radiat. Res., 46, 305-312, (2005).