INTRODUCTION

Giardia lamblia (Giardia intestinalis or Giardia duodenalis) is a flagellated enteric protozoan parasite, unicellular eukaryotic microorganism that commonly causes diarrheal disease throughout the world. It is the most common intestinal protozoan parasite, causing diarrhea in children, especially those with malnutrition or with immunodeficiency ***(Campbell, 1992 and Hakim et al., 2011).***

Although giardiasis is usually self-limited, it can develop into chronic and life-threatening disease. Chronic giardiasis in the early childhood has been significantly associated with malnutrition disorders ***(Gendrel et al., 2003).*** In addition, *Giardia lamblia* is known to be resistant to chlorine at concentrations typically applied for water treatment ***(Carpenter et al., 1999).***

The prevalence of drug resistant strains in a variety of eukaryotic and prokaryotic pathogens has necessitated the need for further study into alternative means of disinfecting agents that have the potential to be more effective than current disinfection options ***(Lenaghan and Sundermann, 2008).***

Although conventional wastewater treatment systems have the capacity to improve effluent quality, yet they are not sufficient to remove all contaminants ***(Von Sperling and Mascarenhas, 2005).***

Disinfection using irradiation technology is of growing interest in the food and water industry since it was demonstrated that radiation is very effective against many organisms ***(Elrifaey et al., 2013).***

Radiation is an innovative method used for disinfection. The use of radiation has been considered from ancient times as the solar disinfection property that was discovered. Recently, the use of ionizing radiation has received much attention due to more efficiency in microbial inactivation without byproducts ***(Al-Ani and Al-Khalidy, 2006).***

In addition, ionizing radiation has more advantages over the weak points of chemical process. The ability of ionizing radiation for converting non-biodegradable substances to more readily degradable ones, besides that this technology never left residues and that is why called a clean technology ***(Tun Tun and Khin khin, 2015).***

Moreover, the typical costs for the treatment of wastewater by radiation are found to be favorably comparable with the other advanced wastewater treatment systems ***(International Atomic Energy Agency "IAEA", 1999).***

In the United States, analysis data on all giardiasis outbreaks reported to the Centers for Disease Control and Prevention for 1971-2011. The 242 outbreaks, affecting approximately 41000 persons, (74.8%) resulted from waterborne. Most (74.6%) waterborne outbreaks were associated with drinking water, followed by recreational water (18.2%) ***(Adam et al., 2016).***

AIM OF THE WORK

The aim of the present work is to evaluate the effect of radioactive Cobalt-60 and 254 nm-UV irradiation on infectivity of *Giardia lamblia* cysts to mice, trying to reduce the probability of *Giardia lamblia* infection in patients, especially immunocompromised.

Plan of the work

1- Isolation of Giardia lamblia cysts from stools of outpatients and inpatients attending laboratories of Ain Shams University hospital and Cairo University hospital with bias to patients with symptoms suggesting giardiasis.

2- Preservation and purification of Giardia cyst using Simple sedimentation technique and Flotation over 1 M sucrose gradient then resuspension in saline for preservation in 4 ◦c.

3- The stored sample was divided into 3 equal parts: The 1st part was irradiated with ultraviolet radiations. The 2nd part was radiated with cobalt 60. And the 3rd part was kept preserved at 4°C without radiation.

4- Preparation of 6 groups of mice divided into:

- **Experimental groups:**

Group I: 10 mice infected with a suspension of G.lamblia cysts irradiated with 0.25 Kilogray of Cobalt-60 ***(Khan, 1992).***

Group II: 10 mice infected with a suspension of G. lamblia cysts irradiated with 254- nm UV rays ***(Li et al., 2007).***

- Control groups:

Group III: 10 mice infected with non-irradiated *Giardia lamblia* cysts.

Group IV: 10non-infected mice that received water irradiated with 0.25 Kilogray of Cobalt-60.

Group V: 10non-infected mice that received water irradiated with 254nm UV rays.

Group VI: 10 non-infected mice, received non-treated water.

5- Then evaluation of the infectivity of *Giardia lamblia* cysts:

1- Stool analysis for the mice on day0, 9, 10, 11. 12 and 21.

2- Duodenal aspiration from mice and examination of the aspirate for the presence of *Giardia lamblia* cysts or trophozoites.

3- Histopathological examination of the small intestine and assessment of grade of inflammation and villus architecture.

REVIEW OF LITERATURE

Historical background

Giardia lamblia (G. lamblia) is a protozoan parasite that was initially discovered by Antonie Van Leeuwenhoek in 1681 while examining his own diarrheal stools under the microscope. The organism was not described until 1859 when Vilem Lamb thought the organism belonged to the genus Cercomonas and named it *Cercomonas lamblia* ***(Nygard et al., 2006).***

It was named after Professor A. Giard of Paris and Doctor F. Lambl of Prague ***(Farthing et al., 2003).***

In 1879, Grassi named a rodent organism now known to be a *Giardia* species, *Dimorphus muris*, apparently unaware of Lambl's earlier description, In 1882 and 1883, Kunstler described an organism in tadpoles that he named *Giardia*, the first time *Giardia* was used as a genus name ***(Garcia, 2007).***

There are several names for the protozoan. The name *G. lamblia* became widely accepted through the 1970s. Since the 1980s, some have encouraged the use of the name *G. duodenalis* and in the 1990s, the name *G. intestinalis* has been encouraged by other investigators, Nowadays, there is no reason to abandon the term *G. lamblia* which has been widely accepted in the medical and scientific literature ***(Garcia, 2007).***

Taxonomy:

According to ***Roberts and Schmidt (2009),*** the taxonomy of *Giardia* is as follows:

Kingdom:	Protozoa
Subkingdom:	Archezoa
Phylum:	Retortamonada
Class:	Diplomonadea
Order:	Diplomonadida
Family:	Hexamitidae
Genus:	*Giardia*
Species:	*lamblia*

Morphology:

G. lamblia is an eukaryotic organism which has a distinct nucleus and nuclear membrane, cytoskeleton, and endomembrane system. In contrast, it lacks nucleoli and peroxisome organelles that are nearly universal in eukaryotes. In addition, *G. lamblia* is anaerobic, lacking mitochondria or any of the components of oxidative phosphorylation ***(Thompson et al., 1993).***

A) Trophozoite:

It colonizes the proximal small intestine and is responsible for the production of diarrhoea and malabsorption ***(Farthing et al., 2008).***

It is tear drop shaped with a pointed posterior end, measuring 10-20 μm in length and 5-15 μm in width, laterally resembles the curved portion of a spoon ***(Garcia, 1997)***. The trophozoite has a convex dorsal surface and a concave ventral surface containing the ventral disc as shown in figure (1). This organelle is unique to *Giardia* and it is a rigid structure consisting of microtubules, cross-bridges attached to microtubules and microribbons which run perpendicularly to both the microtubules and the cross-bridges, The disc contains a variety of cytoskeletal proteins, this proteins give the disc flexibility and allow it to change shape, a process that is thought to be important for attachment ***(Farthing et al., 2008).***

Giardia trophozoites are largely devoid of cytoplasmic organelles, the major structure being multiple ovoid vacuoles which appear to resemble lysosomes, other structures include what may be a primitive Golgi apparatus that is involved in protein sorting, bacterial endosymbionts and a 35-nm double-stranded RNA virus ***(Farthing et al., 2008).***

The dorsal surface apparently provides an area for diffusion of nutrients. There are four pairs of flagella, two

nuclei, that are transcriptionally active containing approximately the same number of genes and the same amount of DNA, two axonemes and two slightly curved bodies called the median bodies ***(Garcia, 1997).***

Motile trophozoites exhibit forward movement during which the organism tends to rotate around its longitudinal axis displaying both a tumbling movement resembling that of a falling leaf and an up and down movement referred to as 'skipping'. Trophozoites can become detached and can subsequently re-attach themselves to the surface of another enterocyte. The cycle of attachment, detachment and subsequent re-attachment may be necessary to compensate for rapid enterocyte turnover and the sloughing of host cells into the intestinal lumen ***(Garcia, 2007).***

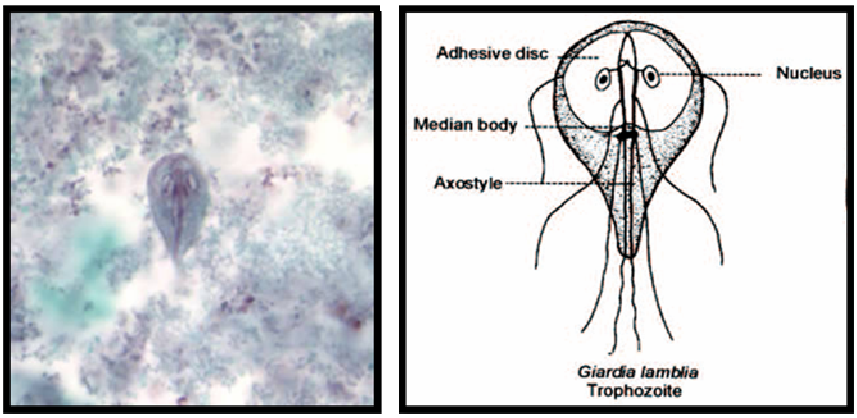

Fig. (1): *Giardia lamblia* trophozoite (Trichrome stained) from "www.dpd.cdc.gov", accessed online on (3/3/2016), *Giardia* trophozoite diagram ***(adopted from Sinnis, 2009).***

B) Cyst:

The stage which is able to exist outside the host in a suitable environment and is the form of the parasite by which Giardiasis is usually transmitted ***(Farthing et al., 2008),*** it may appear rounded or oval, measuring 11-14 μm in length and 7-10 μm in width ***(Huang and white., 2006).***

It contains four nuclei at one pole, axonemes, and median bodies surrounded by a cyst wall (Figure 2) ***(Erlandsen et al., 1990).***

The cyst is covered by a 0.3-0.5 μm-thick cyst wall. which is composed of two layers: outer filamentous layer and an inner membranous layer (further composed with two membranes). The cyst wall is separated from the plasma membrane of the parasite by a peripheral (peritrophic) space ***(Erlandsen et al., 1990).*** The cyst wall is composed of a layer of fibrils arranged as a felt-like web. There is controversy as to whether N-acetyl glucosamine orgalactosamine is the major cyst wall sugar ***(Farthing et al., 2008).***

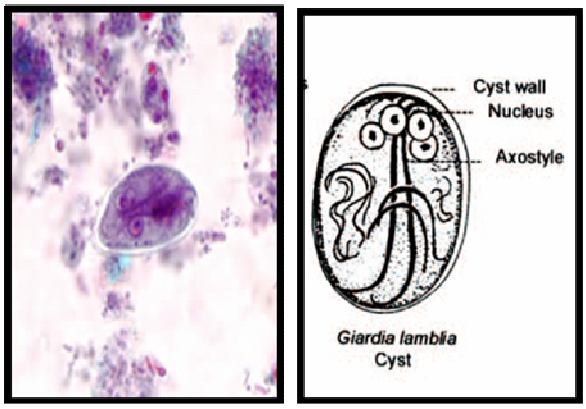

Fig. (2): *Giardia lamblia* cyst (Trichrome stained) from "www.dpd.cdc.gov", accessed online on (3/3/2016), *Giardia* cyst diagram ***(adopted from Sinnis, 2009).***

Life cycle:

Life cycle that alternates between an actively swimming reproductive stage, the trophozoite, and an infective resting stage, the cyst, which is voided in the faeces. It includes two major steps; excystation and encystations (Figure 3). The excystation starts when susceptible host ingests the cysts (infective stage) ***(Garcia, 2007).*** Infection being initiated with as few as 10-100 cysts. Excystation occurs in the proximal small intestine where the trophozoite multiplies ***(Farthing et al., 2008).***

Colonization involves three processes: excystation, attachment to the intestinal epithelium and multiplication ***(Farthing et al., 2008).***

As the cysts pass through the acidic pH regime of the stomach, the low pH and elevated carbon dioxide followed by

slightly alkaline environment of the proximal small intestine induce excystation. The flagella emerge through the cyst wall first, followed by the entire trophozoite ***(Garcia, 2007),*** once trophozoite emerges from the quadrinucleate cyst, it undergoes rapid cytoplasmic division without nuclear division to form two binucleate trophozoites (i.e each cyst gives rise to 2 trophozoites) ***(Roxstrom-Lindquist et al., 2006).***

Trophozoites, the vegetative form attach themselves to the luminal surface of the epithelial cells that line the duodenum and jejunum, and then undergo further division by asexual binary fission ***(Garcia, 2007).***

The final stage of the life cycle is encystation, following exposure of trophozoites to high concentrations of conjugated bile salts and myristic acid at neutral pH. Thus, bile and bile salts may have a dual role in the parasite lifecycle, on one hand promoting growth and multiplication, while at the same time ensuring that the parasite completes its life cycle by encystation ***(Farthing et al., 2008).***

As encystation begins, the trophozoites become rounded, progressively refractile and retract the flagellae into the axonemes. The cytoplasm becomes condensed, and then the cyst wall is secreted and becomes separated from the plasma membrane of the parasite by a peripheral space. As the cyst matures, nuclear, but not cytoplasmic division occurs to

produce the quadrinucleated, mature infectious cyst ***(Ford, 2005).***

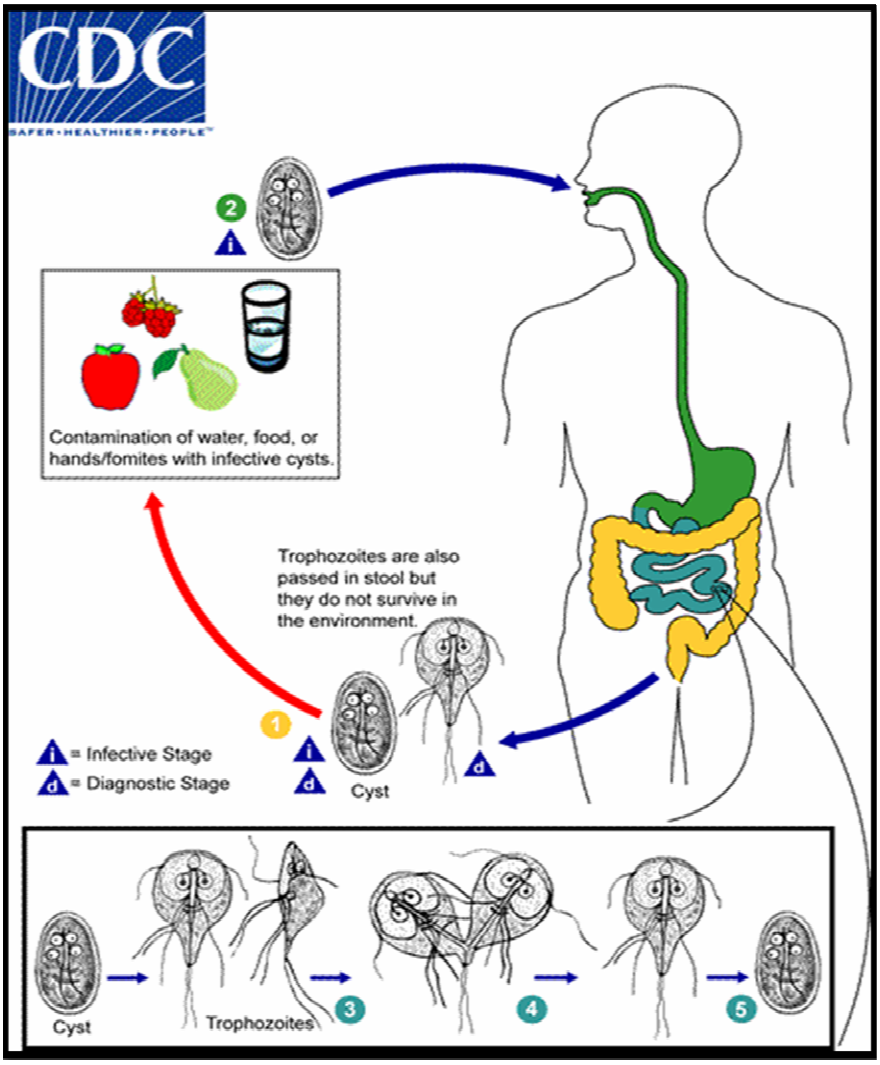

Fig. (3): Life cycle of *Giardia lamblia* from "www.dpd.cdc.gov" accessed online on (3/3/2016)

Epidemiology:

Giardia lamblia is considered the most common protozoal infection in humans; it occurs frequently in both developing and industrialized countries. It is especially prevalent in children in developing countries, where prevalence rate can reach up to 30% ***(Faubert, 2000).*** The prevalence of *Giardia* in stool specimens is 2-7% in industrialized countries as USA and 40 % in developing countries ***(Hakim et al., 2011).***

In Europe, data on giardiasis are collected by 23 countries and are made available by the European Center for Disease Control. Romania reported the highest rates of infection (816.9 per 100,000, sixteen times the EU average), followed by Estonia (34.9 per 100,000) and then Bulgaria (28.7 per 100,000) and Sweden (14.2 per 100,000), The overall notification rate of giardiasis was 58.1 per 100,000, which is extremely high compared to the two major food-borne pathogens, while in New Zealand, giardiasis is notified since 1996, and represents the third most commonly notified communicable disease after campylobacteriosis and salmonellosis. In a recent survey in New Zealand, it was confirmed that giardiasis has one of the highest incidence rates (49.4 per 100,000 population) compared with other developed countries, and this may be related to environmental or social factors ***(Hoque et al., 2004).***

In a recent review of giardiasis in Asia based on 33 studies published in the period 2002-2007 it has been shown that the prevalence varied markedly between studies being higher in urban than in rural areas, among poor communities, slightly higher in males than in females, high prevalence rates were observed in children in Nepal (73.4%), in Thailand (37.7%) and in Malaysia (24.9%) ***(Dib et al., 2008).***

In Africa, the percentage of people expelling *Giardia* cysts was reported to be 17.3% in South Africa ***(Adams et al., 2005),*** 11.7% in Morocco ***(El Kettani et al., 2006),*** 2-11.4% in Ethiopia ***(Gelanew et al., 2007),*** 29% in Sierra Leone ***(Gbakima et al., 2007)*** and 13.9% in Cote d'Ivoire ***(Quihui et al., 2010).***

It has been estimated that the actual prevalence of Giardiasis is 2.97% in the asymptomatic population and 5.84% in the symptomatic population ***(Horman et al., 2004).***

In Egypt, there are previous epidemiological data about *G. lamblia* in humans. ***Zaki et al. (1986)*** found *G. lamblia* in 44% of the population studied in rural zones while ***Shukry et al. (1986)*** in 33% of the people in Cairo and ***Curtale et al. (1998)*** studied faecal samples and detected *G. lamblia* in 24.7% of the samples in Behera Governorate. ***Mahmud et al. (2001)*** found antibodies against *G. lamblia* in samples from infants in Bilbeis.

A recent study conducted in Egypt revealed that the highest prevalence of *G. lamblia* was found in Gharbia, with 44.4% of the faeces positive, followed by Ameria 38.5%, and Kafr El Sheik 25% ***(Foronda et al., 2008).***

In another recent study conducted in Minia district, ***Abdel Hafeez et al. (2012)*** found prevalence of *G. lamblia* among children to be 17.6% and reported that *Giardia* together with *Entamoeba histolytica* are more common than other protozoa in immunocompetent children. On the other hand, ***Abdel Salam (2012)*** found *G. lamblia* in 10% of swimming pool samples in Alexandria.

In Ismailia a study showed that the prevalence of *G. lamblia* was 53% in ruminants and 21% in symptomatic children and infection was not positively correlated with diarrheal symptoms ***(Helmy et al., 2014).***

Abreu-Acosta et al. (2007) have detected differences between the prevalence of protozoa when they used morphological and molecular methods, obtaining higher prevalence with the molecular one. Therefore, prevalence data based on morphological detection most certainly underestimate true prevalence because of its low sensibility ***(Abreu-Acosta et al., 2007).***