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RESEARCH ARTICLE

The effect of hot water extract of *Eucalyptus* on *Giardia lamblia* parasite in vivo

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Abstract

The current study was conducted in the period from February 2012 till February 2013 to investigate the effect of *Eucalyptus* aqueous extract in *G. lamblia* in infected white mice Bulb/c. Flagyl was used in addition to the control solution (physiological solution) as a control groups, the results of the study had been proved the effect of the extract by increasing the concentration without any side effect on the animals of the experiment and the results were as follows:

The *Eucalyptus* extract 3000 mg/kg. is more efficient on *G. lamblia* compared with the Metronidazole and The 2000 mg/kg extract came next where it showed high treatment efficiency notably during the second day and finally the 1000 mg/kg extract came last which is the least effective during the 6^{th} day compare with Flagyl, it was very effective during the fourth day. It was noticed, during a histological study of mice liver and small intestine tissues we notice hypoxia, necrosis, hypertrophy, and a congestion of blood vessels. There are significant differences for the *Eucalyptus* extract and Flagyl also with days treatment in compare with Flagyl.

Introduction

The protozoan, now called *Giardia*, was first described by van Leeuwenhoek in 1681 from his own stool **[7]**, Lambl (1859) had described this same organism as *Cercomonas intestinalis*. The first use of the name *Giardia* for the genus was when Grassi (1879) described *G. agilis* from a tadpole. *Giardia* from humans has been called *G. lamblia* as well as *Lamblia intestinalis* (the latter was especially used in Eastern Europe), but **[21]** determined that *G. intestinalis* is its more appropriate name. Numerous names for what we now call *Giardia* appear in the literature undoubtedly because of the lack of effective communication in earlier times.

Giardia spp. is flagellated protozoan parasites of vertebrates; their infectious stage, the cyst, is transmitted via the fecal–oral route and frequently via water **[35]** .The genus Giardia has been divided into three morphological types: *G. duodenalis* , *G. lamblia* , *G.intestinalis*, *G. muris*, and *G. agilis* **[32].** *G. duodenalis* and *G. muris* have been reported from mammals and birds **[32]** . In the wild, a wide variety of aquatic and semi-aquatic mammals can be a source of waterborne Giardia cysts **[9]**.

Giardiasis is one of the most common protozoan intestinal zoonotic diseases. The pathogen is the most frequently identified etiologic agent of waterborne and food borne outbreaks of intestinal illnesses worldwide **[35]**. Over 44% of the outbreaks in which Giardia was identified as an etiologic agent were food borne, versus 30% and 26% drinking and recreational water related epidemics, respectively **[15**, **25]** . The zoonotic reservoirs for waterborne cysts include aquatic and semi-aquatic mammals, e.g., beavers and muskrats, small rodents, and wild or domestic mammals **[26]**.

Contamination of surface waters is enhanced by adverse weather conditions, which carry cysts from fields containing feces of livestock and wildlife, and cause sewage and waste-related water contamination **[15]**. Human infections manifested by a transient or persistent acute steatorrhea, or intermittent acute diarrhea and abdominal cramps malabsorption, nausea, abdominal distention, malodorous flatulence, and occasional vomiting are associated with weight loss **[35]**.

Giardia infections can be generated by as few as 10 waterborne cysts **[35]**. Giardiasis has a median prepatent period of 14 days, the median incubation time of 8 days, and infections are usually self-limiting. Treatment includes quinacrine metronidazole, tinidazole, or furazolidone **[35]**. Giardia cysts can remain viable in surface water for approximately 2 months **[6]**.

In the water, these cysts gravitate to the bottom of the reservoir and are more easily found in the sediment than in the water **[6]**. Also, sedimentation is the most efficient procedure for the removal of Giardia cysts during drinking water treatment **[30]**. A long term (over 14 months) ecological investigation demonstrated that Giardia sp. Cysts originating from beaver and muskrat colonies settled rapidly to the bottom of slow-moving water reservoirs and contaminated the sediment **[26]**.

Microscopic analyses **[31]** showed that Giardia's nuclei have a semi-open mitosis in which two extranuclear spindles attach to the chromatin through openings at the poles of the nuclear membranes. 374 Emerging Protozoan Pathogens Giardia trophozoites are lipid auxotrophs **[20]**, that is, they do not synthesize their lipid requirements *de novo*. While isoprenylation of proteins probably occurs in Giardia, the complete conversion of mevalonate to isoprenoids and then to cholesterol does not **[24]**. Phospholipids and cholesterol are salvaged from the environment from lipoproteins , cyclodextrins and bile salts **[10, 13, 23]** in its external milieu.

Cyst-wall synthesis in Giardia requires the induction of pathways of protein and carbohydrate (polysaccharide) synthesis. The filamentous portion of the Giardia cyst wall is composed of around 63% of a unique polysaccharide and around 37% proteins **[12]**.

In a Study of **[2]** conducted about the effect of aquatic extracts of *Alliums satvium* to treatment the Giardiasis infection by killing trophozoite phase by effecting on the Cystein proteinases. In other study found that *Alliums satvium* have active materials Allyl alcohol and Allyl mercaptan which had effect against Giardia **[18]**.

Aim of the study:

In this research we study the effect of hot water extract of *Eucalyptus* leaf in different concentrations on the infection of *G. lamblia* in laboratory white mice, as a substitute to Metronidazole which had a side effect when using as treatment for Giardiasis infection.

Material and Methods

Preparation of hot water extract of *Eucalyptus*

Leaf of *Eucalyptus* collected and cleaned, dried and crashed, 100 gm of powder and 200 ml of boiling water mixed in 500 ml beaker for 15 min. and left to 30 min then filtrate the mixer by multi layer of gas and the centrifuge the nomination at 150 rpm for 2h. dried the extract in oven at 40 C°, the we weight the dried extract and preparation many concentrations **[32]**.

Laboratory animals:

15 Swiss white mice Balb/C weight 25 ± 2 gm divided to 5 groups each group include 3 animals were put in iron cages and supplied with especial food in house keeper of Science College /Babylon University and examined daily to confirm the infections of the animals, all animals infected with cyst and trophozoite stage by using stomach tube and waiting for 5 days then we examined the feces of all animals by Direct smear method and after we checked the present of infection for all animals putting with other not infected animals and use sawdust in cages to transfer the infection for all animals **[34]** and waiting for 30 days, then we examined the feces of all animals to examine the infection of *G. lamblia* .

Animals experimental:

All animals' dosage the extracts by using stomach tube with different concentrations as follows:

1. First group dosed with 1000 mg/kg extract.
2. Second group dosed with 2000 mg/kg extract.
3. Third group dosed with 3000 mg /kg extract.
4. Forth group (control) dosed with 30mg/kg Metronidazole.
5. Fifth group (control) dosed with normal saline 0.85%.

We examined the feces of all animals for 24 days to record the rate of cure or healing rate for animals.

Preparation of histological sectioning:

After the period of experimental finished all the animals prepared for anatomy according to **[4]** method of histological sectioning for liver and intestinal.

Result

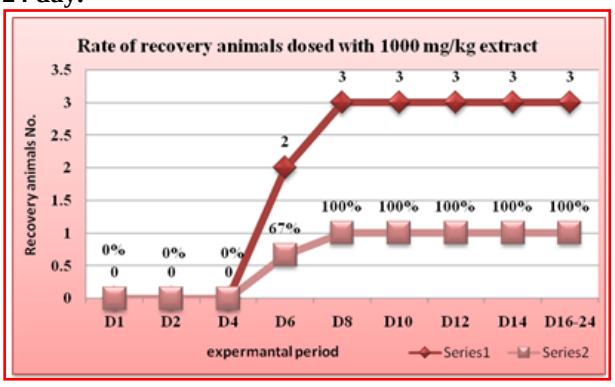
Table (1) and figure (1) shows the number of recovery animals after dosed with 1000 mg/kg hot water extract of *Eucalyptus* , the examination continues for 24 days to record the rate of recovery animals , two animals recover in sixth day with rate 66.6% and in the eighth day we notice that all animals recover in rate 100% ,and no dead animals found and without any side effect on them, the static analysis results shows significant difference (p> 0.05) for recovery animals during 24 day.

Table (1): the number and percentage rate of recovery animals which dosed with 1000 mg/kg hot water *Eucalyptus* extract during 24 day.

Days No.	Recovery animals No.	Dead No.	Recovery percentage %	Side effects
1st day	0	0	0	Not found
2ed day	0	0	0	=
4th day	0	0	0	=
6th day	2	0	66.6	=
8th day	3	0	100	=
10 day	3	0	100	=
12 day	3	0	100	=
14 day	3	0	100	=
16-24 day	3	0	100	=

F= 9.14, for days F (0.005)=5.60
F= 7.12, for concentration F (0.005)=5.60

Figure (1): percentage rate of recovery animals dosed with 1000 mg/kg *Eucalyptus* extract during 24 day.



The results shows 2 animals recovery in the fourth day (66.6%) after dosed with 2000 mg/kg *Eucalyptus* extract and at the sixth day we notice all the animals are recovered (100%) and no dead animals found and without any side effect on them, the static analysis results shows significant difference (p> 0.05) for recovery animals during 24 day as in (table 2 and figure 2).

Table (2): the number and percentage rate of recovery animals which dosed with 2000 mg/kg hot water *Eucalyptus* extract during 24 day.

Days No.	Recovery animals No.	Dead No.	Recovery percentage %	Side effects
1st day	0	0	0	Not found
2ed day	0	0	0	=
4th day	2	0	66.6	=
6th day	3	0	100	=
8th day	3	0	100	=
10 day	3	0	100	=
12 day	3	0	100	=
14 day	3	0	100	=
16-24 day	3	0	100	=

F= 9.14, for days F (0.005) =5.60
F= 7.12, for concentration F (0.005) =5.60

Figure (2): percentage rate of recovery animals dosed with 2000 mg/kg of *Eucalyptus* extract during 24 day.

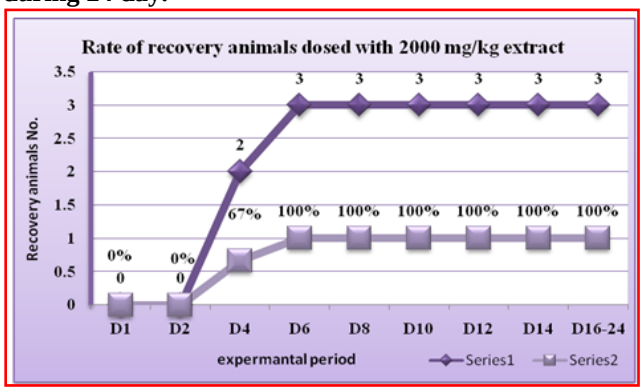


Table (3) and figure (3) shows the percentage rate of recovered animals after dosed with 3000 mg/kg hot water *Eucalyptus* extract, we notice that all animals recovered in second day (100%) no dead animals found and without any side effect on them, the static analysis results shows significant difference (p> 0.05) for recovery animals during 24 day.

Table (3): the number and percentage rate of recovery animals which dosed with 3000 mg/kg hot water *Eucalyptus* extract during 24 day.

Days No.	Recovery animals No.	Dead No.	Recovery percentage %	Side effects
1st day	0	0	0	Not found
2ed day	3	0	100	=
4th day	3	0	100	=
6th day	3	0	100	=
8th day	3	0	100	=
10 day	3	0	100	=
12 day	3	0	100	=
14 day	3	0	100	=
16-24 day	3	0	100	=

F= 9.14, for days F (0.005) =5.60
F= 7.12, for concentration F (0.005) =5.60

Figure (3): percentage rate of recovery animals dosed with 3000 mg/kg of *Eucalyptus* extract during 24 day.

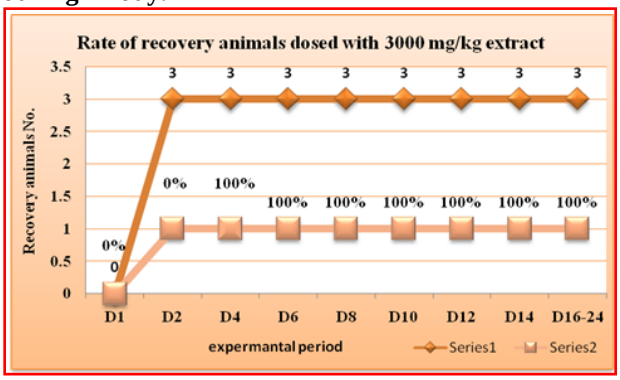


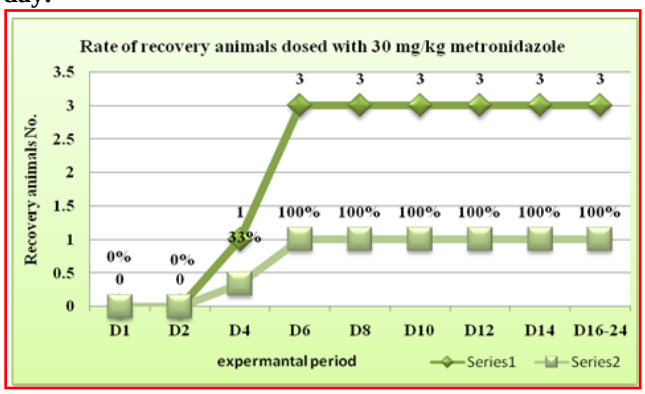
Table (4) and figure (4) shows percentage rate of recovered animals after dosed with 30 mg/kg Metronidazole , we notice one animal recovered in fourth day (33.3%) and in the sixth day all animals recovered 100% , and no dead animals found and without any side effect on them, the static analysis results shows significant difference (p> 0.05) for recovery animals during 24 day.

Days No.	Recovery animals No.	Dead No.	Recovery percentage %	Side effects
1st day	0	0	0	Not found
2ed day	0	0	0	=
4th day	1	0	33.3	=
6th day	3	0	100	=
8th day	3	0	100	=
10 day	3	0	100	=
12 day	3	0	100	=
14 day	3	0	100	=
16-24 day	3	0	100	=

Table (4): the number and percentage rate of recovery animals which dosed with 30 mg/kg Metronidazole during 24 day.

F= 9.14, for days F (0.005) =5.60
F= 7.12, for concentration F (0.005) =5.60

Figure (4): percentage rate of recovery animals dosed with 30 mg/kg of Metronidazole during 24 day.



In the fifth group the infected animals dosed with normal saline and we found that all animals stay infected and without any recovery rate, table (5) shows the study group which dosed with *Eucalyptus* extract and flagyl and normal saline with continues examination for 24 day.

Histological study shows tear in intestinal lumen shows expansion and destruction of villi and Polymorphic infiltration, destruction epithelial cells lining the villi and debris, ls in the fourth group of infected animals dosed with 30mg/kg Flagyl **(**Figure 5).

Figure (5): section in the small intestine of infected animals dosed with 30 mg/kg of Metronidazole , shows: A- expansion and destruction of villi. B- Polymorphic infiltration. C - Destruction epithelial cells lining the villi. D- Debris (H. & E.100X).

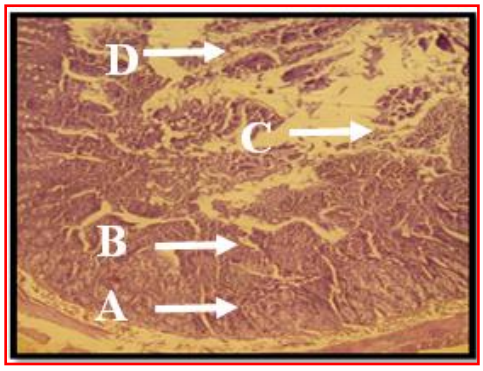


Table (5): the total number of infected animals by *G. lamblia* which dosed with *Eucalyptus* extract and Flagyl and normal saline during 24 days.

day / dose Con.	day 1	2	4	6	8	10	12	14	16	18	20	22	24
1000 mg/kg extract	3 infected mice	3 infected mice	3 infected mice	1 infected mice	0 infected mice	=	=	=	=	=	=	=	=
2000 mg/kg extract	3 infected mice	1 infected mice	0 infected mice	=	=	=	=	=	=	=	=	=	=
3000 mg/kg extract	3 infected mice	0 infected mice	=	=	=	=	=	=	=	=	=	=	=
30 mg/kg flagyl	3 infected mice	3 infected mice	2 infected mice	0 infected mice	=	=	=	=	=	=	=	=	=
Normal saline	3 infected mice	3 infected mice	3 infected mice	=	=	=	=	=	=	=	=	=	=

Figure (6) appears section in the tissue of liver for the infected animals dosed with 30 mg/kg Metronidazole notes hemorrhage and necrosis in liver cords also central infiltration in liver cords.

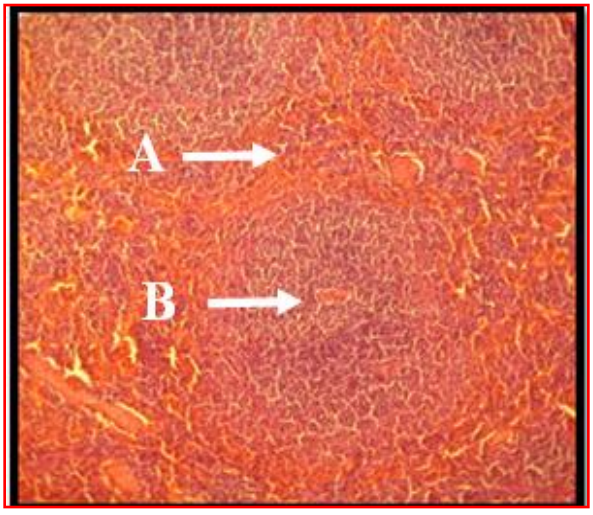


Figure (6): section in the liver tissue for infected animals dosed with 30 mg/kg Metronidazole , shows: A- Hemorrhage between kupffer cells and necrosis in liver cords. B- Central infiltration in liver cords (H. & E.100X).

In figure (7) shows cyst stage of *G. lamblia* preparation by iodine stain, and figure (8) appear trophozoite stage preparation by Giemsa stain, isolated form feces of infected animals.

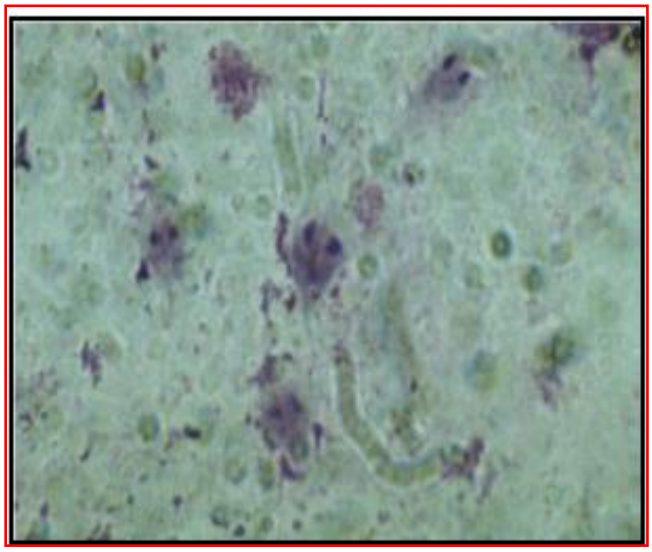


Figure (7): cyst stage of *G. lamblia*

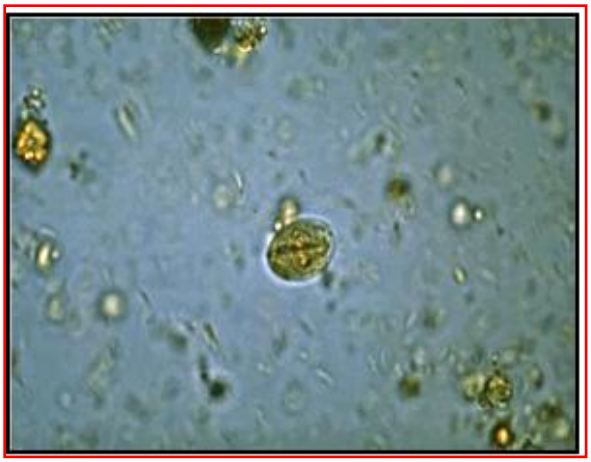


Figure (8): trophozoite stage, Giemsa stain, iodine stain, (40x)

Discussion

We notice from the histological study tear in small intestinal lumen and necrosis expansion and destruction of villi and Polymorphic infiltration, destruction epithelial cells lining the villi and debris , also in the tissue of liver of infected animals dosed with 30 mg/kg Metronidazole notes hemorrhage and necrosis in liver cords also central infiltration in liver cords, maybe these changes due to the infection of *G. lamblia* which cause tearing the lumen of small intestine by adhesive disks also the accumulation of toxic material in hepatic cells cause congestion and deficiency in cells functions **[32]** or the dose of metronedazole had a very strong effect on infected animals and cause these changes in the tissues **.**

The research results show that the effect of 3000 mg/kg hot water *Eucalyptus* extract is more capability to cure or recovery the infected animals, while the doses of 1000 and 2000 mg/kg less efficiency , maybe the concentration of extract isn't enough to recovery animals in short time , in the dose of 30mg Metronidazole we notice the animals stay infected in first day and second day and two in fourth day and all animals recovery in sixth day .

In the event of infections, several prescription drugs are available to treat giardiasis. The cure rates of antigiardia compounds vary by the study, and range from 80 to 100%. Metronidazole (commercial name Flagyl) is recommended by the World Health Organization for chemotherapy. It is very effective and is the most commonly used drug in adult patients in the United States; however it has an unpleasant flavor and children do not tolerate it very well. As tolerance may be a problem in pregnant mothers and young children, furazolidone is recommended in expectant women **[8].** Although nausea or vomiting may still occur **[1] ,**Furazolidone or nitoxozanide may be used for treating pediatric Giardiasis. Additional drugs include tinidazole, a compound chemically related to metronidazole, and quinacrine. These two products are not available in the United States, although tinidazole is frequently used in other countries. Quinacrine is useful in the management of difficult cases and can be obtained through Panorama Pharmacy, Panorama City, CA **[28]**. Other products used to treat Giardiasis include paramomycin and albendazole and metronidazole-related compounds such as ornidazole and secnidazole **[11].**

Dependent cause that explain the activity of *Eucalyptus* extract in prevent or killing the infection of Giardiasis is the active materials such as ketone , florid, saponins, tannins , carbohydrates,flavonoid, oil, polar material soluble in water and many other compounds , therefore *Eucalyptus* consider a very important medical plant **[38].**

Tannin could interaction with the plasma membrane of the parasite and destroy it by influence the lipids and membrane proteins and as a result the parasite loss his ability to grow or doing the normal activities , also these materials inter the plasma membrane and inactivate the active sites of many enzymes which essential for living organism **[3].**

Other activity for *Eucalyptus* extract is ability to increase the movement of intestine and this make hard to parasite to adhesive to epithelial cells of lumen intestine, and decrease the infection of *G. lamblia* **[32].**

Leaf of *Eucalyptus* contain a high concentrations of iron and this element had important role in killing *G. lamblia* because it's a toxic substance but we can use it in medical benefits **[14].**

Extract concentration and crude matter in the tissues of organism depend on some physiological factors such absorption area and feeding quantity and rate of metabolism and excretion rate and extract function and active sites and this produce differences in parasite stages responses for extract **[29]**.

Referred **[17]** to the activity of tannins ability to interaction with proteins inside living organism and disorder the metabolism functions by influence the nitrogen and amino acid which consider very important to organism live.

[2] study the effect of aquatic extracts of *Alliums satvium* to treatment the Giardiasis infection by killing trophozoite phase by effecting on the Cystein proteinases. In other study found that *Alliums satvium* have active materials Allyl alcohol and Allyl mercaptan which had effect against Giardia **[18].**

Waterborne outbreaks of Giardiasis are a major public health problem in many industrialized nations, including the United Kingdom, Sweden, Canada, and

the United States **[22, 27].** Human sewage has been considered a source of Giardia cysts contamination in water. In Canada and Italy, a high prevalence (73–100%) of Giardia cysts was reported in raw sewage samples **[5, 19, 35].** The public health importance and contamination sources of Giardia cysts found in water, however, are largely unclear, because very few studies have been carried out to genetically characterize the *Giardia* cysts in water. Nevertheless, *G. intestinalis* cysts of assemblage have been identified in a few clams collected from the Rhode River, a Chesapeake Bay subestuary in Maryland **[15].**

Giardia cysts are very resistant to conventional water treatment, such as chlorination and ultraviolet irradiation. For large water systems, sand filtration or a similar method for physical removal, in addition to an effective disinfection treatment can be a successful water treatment option. For individual water supplies it is advisable to have the water tested prior to selecting a treatment option. Frequently used are combinations of filtration disinfection, and occasionally, reverse osmosis. To verify the efficacy of water treatment systems or point of use devices, visit the Web site of the National Sanitation Foundation **[37].**

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