

The Therapeutic Effect Of Eucalyptus Microtheca Against The Giardiasis Induced Renal Damage In Male Rats

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ABSTRACT

The current study was designed to show the role of aqueous crude extracts against the *Giardia lamblia*. The study used 20 rats (Adult male) that distributed into four groups; the control group. Second group rat infected with (1X10³ cell/ ml) *G. lamblia*. The third group infected rat was treated with (100mg/ml) aqueous extract for four weeks. The fourth group infected rat was treated with (250mg/ml) aqueous extract for four weeks. The results show a highly significant elevate (P < 0.05) in levels of MDA with a significant (P < 0.05) reduction in levels of catalase in infected rats compared with the control group. The results of treated rats show non-significant (P < 0.05) changes in all parameters compare with the control group when using aqueous crude extracts. About the histological changes, in the second group, kidney sections show damage glomerulus, slough endothelial of tubules, lymphocytes infiltration, thickening wall of blood vessels, and fibroblast. In treated groups (100mg & 250mg), show semi-normal glomerulus and urinary tubules. So, an aqueous extract of *E. microtheca* show a high efficacy role against *G. lamblia*.

Keywords: *G. lamblia*, Eucalyptus microtheca; oxidative stress; kidney.

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INTRODUCTION

The plant called Eucalyptus is well known for its bioactive compounds and contents [1] and is a big source for different unique secondary metabolites like essential oils, glycosides, saponins, alkaloids, and flavonoids that demonstrate a different of biological activities [2-4], Terpenoids, that form most of the essential oil giving eucalyptus foliage its characteristic smell [5]. certain species of Eucalyptus are utilized for feverish cases (malaria, cholera, and typhoid) and problems of skin like the wounds, burns and the ulcers [6], Anticancer properties, antifungal, anti-inflammatory properties [7], and antioxidant properties [8] have been attributed to the leaf extracts of Eucalyptus. *Giardia duodenalis* (syn. *G. intestinalis*, *G. lamblia*) is one of the most dangerous intestinal protozoan flagellates of the man. *Giardia* species life cycle is very simple and it is inclusive of two active forms; trophozoite and cystic stage. *G. lamblia* transport via the fecal-oral route through direct or indirect by ingestion of the infectious cysts [9]. The period of incubation varies from 9 to 15 days after cysts ingestion. Infection symptoms are different from the absence of the symptoms to acute watery diarrhea, epigastric pain, nausea, and weight loss [10-11]. So, the current study was designed to show the role of aqueous crude extracts against the *Giardia lamblia*.

MATERIALS AND METHODS

Animal model

Albino male rats, (4-6 months old and 175-225 gm body weight) were housed in special cages, and maintained under laboratory conditions; temperature (22 ±2) and light (14 hour light and 10 hours dark), standard food and tap water were given to rats.

Purification and enumeration of *G. lamblia* cysts

G. lamblia cysts were purified with some modifications to the original method [12]. Cysts of *G. lamblia* enumerate by using a chamber called Neubauer under the oil objective lens. The sample was diluted by using normal saline to (103) cyst for each rat.

Eucalyptus aqueous crude extracts

Aqueous crude extracts of leaves of eucalyptus were prepared to test against Amoebic dysentery. The method of [13] was used to process aqueous extract. Stock solutions of aqueous extract of eucalyptus were prepared by dissolving 1 g of dried aqueous extract in 10 ml of sterilized distilled water and no further concentrations were made.

Experimental design

Twenty rats were used in this work and after that divided as follow:

- A. Control group received a standard diet.
- B. Second group rat administrated with (dose 10³ cell/ ml.) *G. lamblia*.
- C. Infected rats were treated with 100mg of aqueous crude extracts for four weeks.
- D. Infected rats were treated with 250mg of aqueous crude extracts for four weeks.

Oxidative agents

Serum MDA (malondialdehyde), was measured in this study according to the colorimetric reaction by using thiobarbituric acid (TBA) [14]. S. Catalase was determined by using the Biovision-USA kits procedure.

Histology processing

Kidney species were collected and fixed with 10% formalin, processed by paraffin method, cut at six micrometers in thickness by microtome device, and stain

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step done by using Hematoxylin and Eosin (H&E) [15]. Sections were diagnosed by using the optical microscope.

Statistical analysis

Current data were analyzed by using a program known as Minitab (statistical program). A statistical change between the group's means was analyzed using a one-way analysis of variance.

RESULTS

Oxidative stress

MDA levels show a significant ($P < 0.05$) increase in the second group compare with the control group as shown in figure (1). Where, catalase levels show a significant ($P < 0.05$) decrease in the same group as shown in figure (2). After treatment with aqueous extract, MDA and catalase levels show non-significant ($P < 0.05$) changes in the third and fourth groups compare with the control group as shown in figures (1-2).

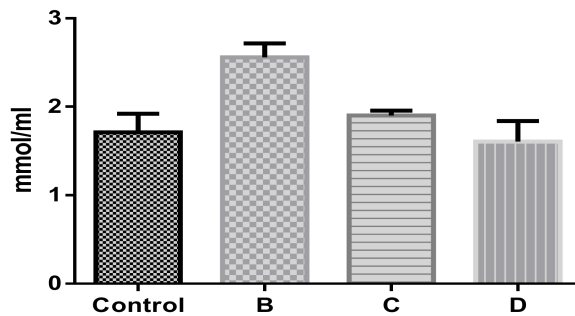


Figure (1): Levels of MDA in serum of all groups

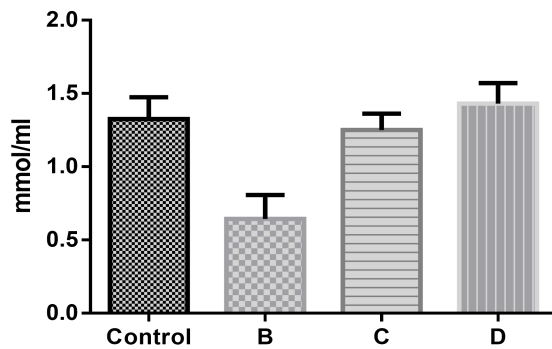


Figure (2): Levels of catalase in serum of all groups

Histological study

The sections of the control group show the normal structure of glomerulus and urinary convoluted tubules (fig. 3). In an infected group, the kidney sections demonstrated damage glomerulus; damaged tubules, lymphocytes infiltration, and fibroblast were present (fig.

4). In treated groups (C & D), kidney tissue show good recovery, especially in the 250 mg, treated group, whereas the glomerulus and tubules present semi-normal shape (fig: 5-6).

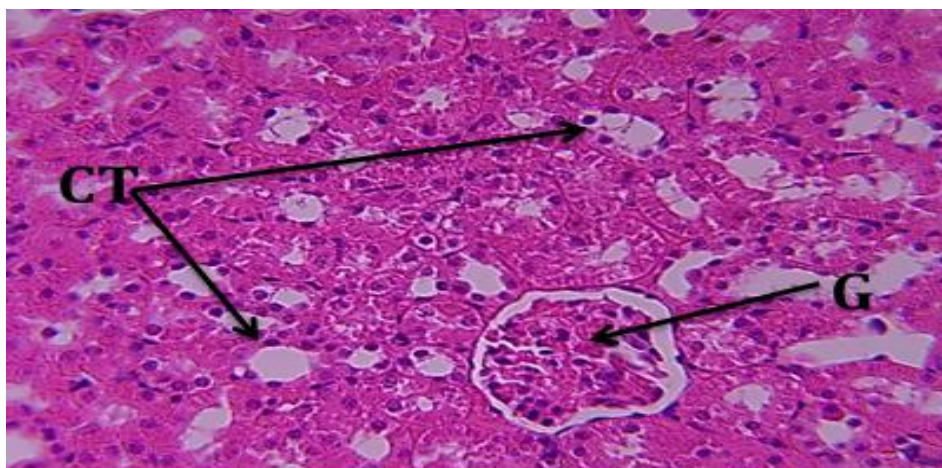


Figure (3): kidney of control group show normal structure of glomerulus (G) and convoluted tubules (CT) H&E

X400.



Figure (4): kidney of infected group show damage glomerulus (DG), slough endothelial of tubules (SE), lymphocytes

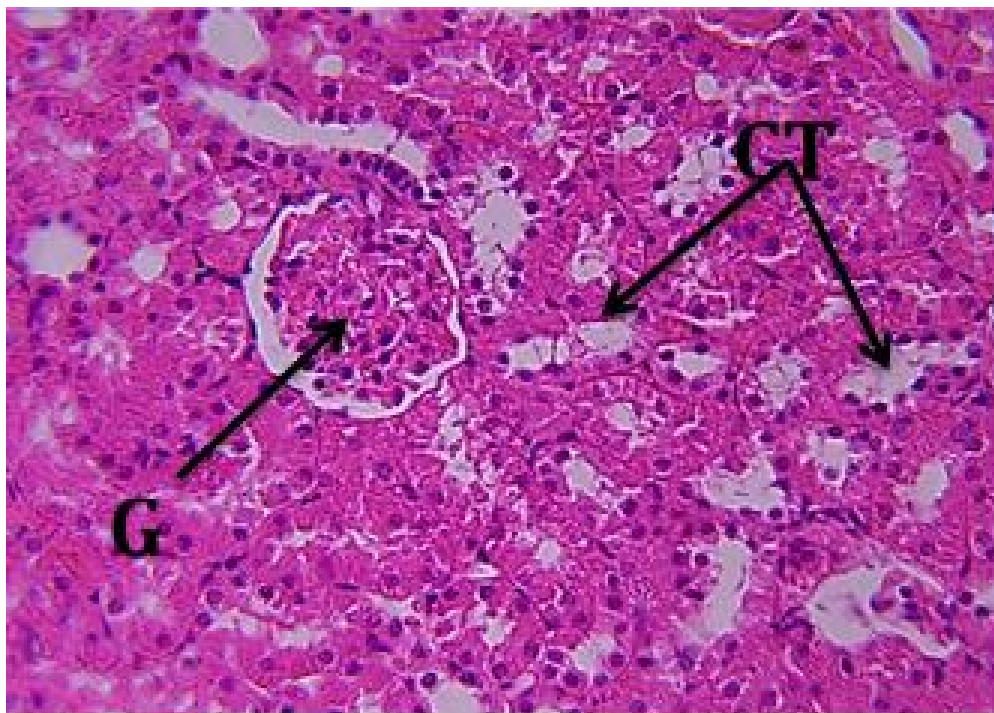


Figure (5): kidney of treated group (150mg/kg) show glomerulus (G) and convoluted tubules (CT) H&E X400.

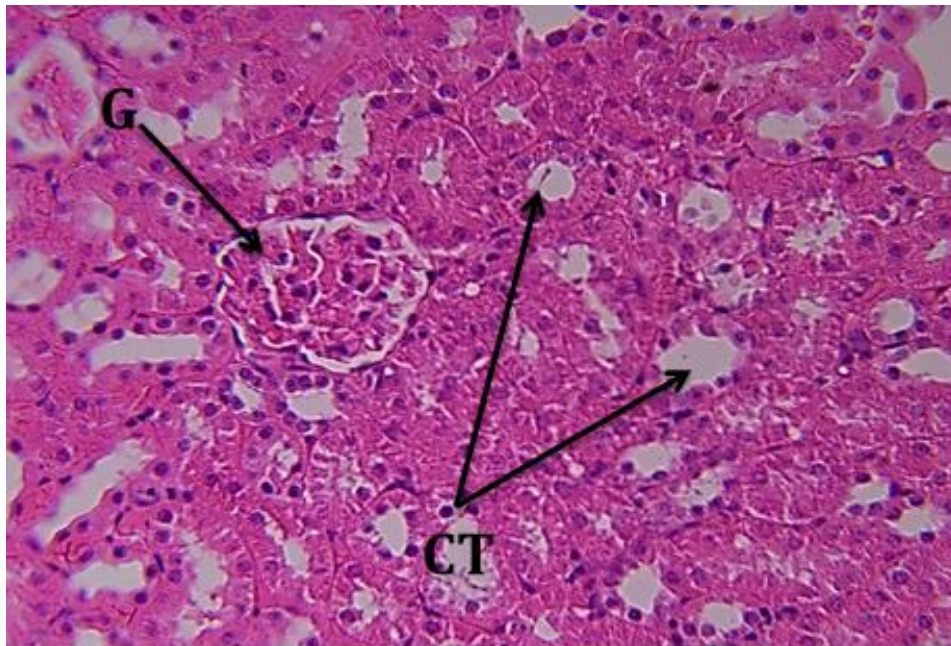


Figure (6): kidney of treated group (250mg/kg) show glomerulus (G) and convoluted tubules (CT) H&E X400.

DISCUSSION

The present results showed that the serum levels of MDA were significantly increased and catalase levels show a significant ($P < 0.05$) decrease in the giardiasis infected rats compared to control groups. The decrease of catalase level and MDA level was significantly increased in the serum of infected rats can give an idea of oxidative damage. The changes level of these parameters may be due to an increase in its catabolism [16]. It may be due to the resistance of the parasite to phagocytosis by increasing the free radical and this leads to a decrease in the level of catalase in the serum of infected rats [17]. About the histological changes in the kidney of the infected group may be due to the Giardia consumes oxygen to a certain extent and produce toxic oxygen derivatives. Furthermore, these organisms are highly susceptible to exogenous reactive oxygen metabolites, such as hydrogen peroxide (H_2O_2) [18] that lead to damage in cells of the kidney. After treatment with aqueous extract, MDA and catalase levels and histological changes back to normal state compare with a control group that may back to several unique secondary metabolites such as essential oils, glycosides, saponins, alkaloids, and flavonoids that found in *Eucalyptus microtheca* [2-4]. Where, flavonoids are the metabolites responsible for the anti-giardia activity exhibited by the methanol extract of *A. scabra* leaves [19], which explain the role of *Eucalyptus microtheca* extract in the treatment that kidney histological damages in the present study. Also, in other study demonstrated that the flavonoids; Bruceantin, (-)-epicatechin, b-sitosterol, b-sitosterol 3-O-b-D-glucopyranoside, hyperin, kaempferol, narcissin, quercetin, and rutin are examples of compounds isolated from distinct plant species whose activity has been examined in vitro against different strains of *Giardia lamblia* by various authors [20-23] that explain the role of *Eucalyptus microtheca* extract in the current study.

CONCLUSION

The outcomes of this study showed a high significant elevate in levels of MDA with a significant reduction in levels of catalase in infected rats compared with the

control group. While the results of treated rats show non-significant changes in all parameters compare with the control group when using aqueous crude extracts. About the histological changes, in the second group, kidney sections show damage glomerulus, slough endothelial of tubules, lymphocytes infiltration, thickening wall of blood vessels, and fibroblast. In treated groups (100mg & 250mg), show semi-normal glomerulus and urinary tubules. Further studies are required to examine more specific inflammatory markers in correlation with GIT complications. Finally, *E. microtheca* is considered the worth anti-parasitic treatment against giardiasis.

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