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STUDY THE ROLE OF TITANIUM DIOXIDE (TIO2) NANOPARTICLES AGAINST THE TOXICITY OF *Echinococcus granulosus* IN ADULT ALBINO MALE RATS

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ABSTRACT

The present study was designed to show the role of green Tio_2 nanoparticles against toxicity of *Echinococcus granulosus*. The present study used 16 adult albino male rats that distributed randomly to following groups (each group consist 5 rats); control group received ad libidium, second group injected with 2,5 X 10^3 of *Echinococcus granulosus* protoscolices third group injected with protoscolices and treated with 50mg/kg green Tio_2 nanoparticles, fourth group injected with protoscolices and treated with 100mg/kg green Tio_2 nanoparticles. The results show high significant increased ($P < 0.05$) in levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) in group injected with protoscolices compared with control group. Oxidative stress factor in group injected with protoscolices show significant increased ($P < 0.05$) in levels of MDA (malonedialdehyied) and significant decreased ($P < 0.05$) in levels of glutathione (GSH) and catalase compared with control group. While, after used green Tio_2 nanoparticles with *Echinococcus granulosus*, the results showed non-significant changes ($P < 0.05$) in liver functions and MDA, GSH and catalase also showed non-significant changes ($P < 0.05$) compared with control group. It was concluded that green Tio_2 nanoparticles has been potential role against toxicity of *Echinococcus granulosus* in male rats.

INTRODUCTION

Titanium dioxide (TiO2) Nanoparticles possess interest in a different applications such as gas sensor, nanomedicine, photocatalyst, dye-sensitized solar cells [1-4] and antibacterial agents [5]. Titanium dioxide (TiO2) Nanoparticles are synthesis and produced by a different techniques ranging from simple methods including mechanical, chemical and vacuum methods, including variants of chemical and physical vapor deposition methods [6], or also by biological synthesis [7]. Eruca sativa (Rocket) is important a medicinal plant with many different properties, including strong aphrodisiac effects [8]. Several studies found that the presence of vitamin C, carotenoids., flavonoids including luteolin, appiin, and glucosinolates the precursors of sulfaraphene and isothiocyanates [9-10] and volatile oils like phellandrene , apiole and myristicin [11-12] Echinococcus parasite are responsible of hydatid cyst (HC). It is a parasite which causes a lot of economic and social problems [13]. Theinfection is made by eating parasite eggs through the mouth.

Potoscoleces phaseare the infective stage of the Echinococcus spp. in definitive host, but they are also able to differentiate asexually into secondary cysts are released in host by accidental rupture of the primary cyst [14].

MATERIALS AND METHODS

Animal model

In this study twenty adult male albino rats, (wt 200-250 gm with age5-8 month) obtained from Technical college/ North Technical University, and kept on standard pellet diet for two weeks to insure its normal and there aren't any infection.

Plant Extraction

leafs of Eruca sativa were obtain from local market. Then, fine powder was obtain and then extracted with ethanol using sahxulate apparatus. The extracts of the leaf was evaporated to dryness in a rotary evaporator at 40ºC. T. The filtrate autoclaved at 121ºC and 15 lb pressure for 20 min.[15].

TiO_2 nanoparticles synthesis

To prepared and synthesis TiO2 nanoparticles, dissolve 1.0 N of Titanium Chloride (TiCl4) in 100 ml of distal water. Then, added leaves extract . The mixture was leaved to stirring for 4 hours. In this process TiO2 nanoparticles were formed, then separate these nanoparticles using filter paper and washed the materials with water to remove the by-products. The nanoparticles were dried at 100°Cfor overnight and storage until using [16].

Echinococcus granulosus

hydatid cysts were collected and obtained from infected sheep livers. They were put in plastic bags, and transported to the Department of Microbiology , College Technical , North Technical University, where protoscolices were isolated from livers according to [17] method . Protoscolices indicates the fertility of hydatid cyst and it's were counted according to method cited by [18]. The viable protoscolices for parasite were counted in 1ml from supernatal based on the formula : Viability in 1 ml = number of protoscolices in (10 µl) × 100.

Experimental design

sixteen adult male albino rats were used and divided as follow (each group consist four rats):

I. Rats were received standard pellet diet only for seven days and then killed.

II. Rats injected with protoscolices, and then killed.

III. Rats injected with protoscolices and treated with 50mg/kg TiO_2 NPs for month, and then killed.

IV. Rats injected with protoscolices and treated with 100mg/kg TiO_2 NPs for month, and then killed.

Prepare of blood solution

The blood collecting from rats by cardiac puncher, under anesthesia, and put in test tubs. After clotting, the tubes were centrifugation for 10 min to obtain sera. The serum was taken and stored by deep freezing until used.

Homogenization

liver samples were removed immediately and the put in glass dish contents 0.9% NaCl buffer for washing and removed the blood. To oxidative stress factors determination, 10% from organ weight was dissolved with buffer (PH 7.4) and the organ tissue was crashed by use ceramic mortar. Then mixture was centerfigation for 10 min. Supernated was taken and stored by deep freezing until used [19].

Measurements

ALT, AST & ALP

Serum ALT, AST & ALP were measured by technique according to the instructions of manufacturer company kit (Randox).

Plasma Peroxidation levels (MDA)

MDA (malonedialdehyied), was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer [20].

Glutathione (GSH) and Catalase

GSH level estimated bymixed 2.3 ml buffer with 0.2ml of the sample and then added 0.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture was analyzed by spectrophotometer [21]. Catalase was measured by using the procedure of Biovision-USA kits.

Statistical analysis

The Data were analyzed using a statistical Minitab program. A statistical difference between the means of the experimental groups was analyzed using one way analysis of variance (ANOVA).

RESULTS

Liver function tests

ALT levels (72.46 ± 5.18), AST (81.24 ± 6.29) and ALP (95.3 ± 6.12) in rats injected with protoscolices show high significant increased ($P < 0.05$) compared with control rats (17.4 ± 2.15, 19.45 ± 2.29 and 57.8 ± 3.32 respectively). The levels of ALT (29.18 ± 1.33), AST (28.21 ± 3.45) and ALP (68 ± 4.52) in rats injected with protoscolices and received 50mg/kg TiO_2 NPs show high significant increased ($P < 0.05$) compared with control rats. While, the levels of ALT (15.34 ± 4.23), AST (20.2 ± 1.73) and ALP (68.75 ± 5.3) in rats injected with protoscolices and received 100mg/kg TiO_2 NPs show no significant decreased ($P < 0.05$) compared with control rats as shown in table (1).

Table (1): The levels of ALT, AST and ALP in serum

Parameters / Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
I	17.4 ± 2.15 c	19.45 ± 2.29 c	57.8 ± 3.32 c
II	72.46 ± 5.18 a	81.24 ± 6.29 a	95.3 ± 6.12 a
III	29.18 ± 1.33 b	28.21 ± 3.45 b	68 ± 4.52 b
IV	15.34 ± 4.23 c	20.2 ± 1.73 c	68.75 ± 5.3 c

Note: same letters mean non-significant changes and different letters mean significant changes.

Oxidative stress parameters (MDA, GSH and catalase)

The levels of MDA (2.34 ± 0.13), GSH (0.307 ± 0.016) and catalase (1.12 ± 0.1) in rats injected with protoscolices show high significant changes ($P < 0.05$) compared with control rats (1.65 ± 0.21, 0.454 ± 0.031 and 1.12 ± 0.1 respectively). The levels of MDA (1.76 ± 0.21), GSH (0.423 ± 0.034) and catalase (0.83 ± 0.09) in rats injected with protoscolices and received 50mg/kg TiO_2 NPs show high significant changes ($P < 0.05$) compared with control rats. While, the levels of MDA (1.51 ± 0.09), GSH (0.432 ± 0.056) and catalase (1.08 ± 0.07) in rats injected with protoscolices and received 100mg/kg TiO_2 NPs show no significant changes ($P < 0.05$) compared with control rats as shown in table (3).

Table (3): The levels of MDA, GSH and catalase in serum

Parameters / Groups	MDA (mmol/l)	GSH (mol/l)	Cata (mmol/l)
I	1.65 ± 0.21 b	0.454 ± 0.031 a	1.12 ± 0.1 a
II	2.34 ± 0.13 a	0.307 ± 0.016 b	0.61 ± 0.04 c
III	1.76 ± 0.21 b	0.423 ± 0.034 a	0.83 ± 0.09 b
IV	1.51 ± 0.09 b	0.432 ± 0.056 a	1.08 ± 0.07 a

In liver

The levels of MDA (1.33 ± 0.12), GSH (0.13 ± 0.03) and catalase (0.37 ± 0.01) in rats injected with protoscolices show high significant changes ($P < 0.05$) compared with control rats (0.87 ± 0.09, 0.23 ± 0.05 and 0.67 ± 0.03 respectively). The levels of MDA (1.09 ± 0.02), GSH (0.18 ± 0.02) and catalase (0.54 ± 0.08) in rats injected with protoscolices and received 50mg/kg TiO_2 NPs show high significant changes ($P < 0.05$) compared with control rats. While, the levels of MDA (0.91 ± 0.04), GSH (0.24 ± 0.08) and catalase (0.63 ± 0.07) in rats injected with

protoscolices and received 100mg/kg TiO_2 NPs show no significant changes ($P < 0.05$) compared with control rats as shown in table (4).

Table (4): The levels of MDA, GSH and catalase in liver

Parameters / Groups	MDA (mmol/l)	GSH (mol/l)	Cata (mmol/l)
I	0.87 ± 0.09 c	0.23 ± 0.05 a	0.67 ± 0.03 a
II	1.33 ± 0.12 a	0.13 ± 0.03 c	0.37 ± 0.01 c
III	1.09 ± 0.02 b	0.18 ± 0.02 b	0 .54 ± 0.08 b
IV	0.91 ± 0.04 c	0.24 ± 0.08 a	0.63 ± 0.07 a

DISCUSSION

The result of present study as previously showed increased in the levels of liver functions when rats administrated with *Echinococcus granulosus* protoscolices, same finding was reported by Khudhair et al (2014) who referred that *Echinococcus granulosus* lead to elevated liver function parameters [22]. Also the results of present study may back to the toxic effects of *Echinococcus granulosus* protoscolices on liver tissue, where its lead to lymphocytes infiltration, degenerative changes, necrosis of hepatocytes and congestions [23]. About oxidative stress and antioxidant factors, the results showed decreased in levels of antioxidant factors and increased in MDA levels in rats administrated with *Echinococcus granulosus* protoscolices, these results is in agreement with who found that the glutathione levels in patients with *Echinococcus granulosus* show significant decreased compare with healthy subjects [24]. Also, Koltas et al. (2006) referred that the infection with *Echinococcus granulosus* lead to eleveated the levels of MDA,

where found the mean +/- SD of MDA levels of patients with *Echinococcus granulosus* [25] that is in agreement with results of present study. About the green nanoparticles, Biosynthesis of nanoparticles by a green method has more advantages over physical and chemical methods as well as it is cost effective and eco-friendly and does not require use of pressure, temperature, toxic chemicals and energy.. The nanoparticles synthesis process has diverse applications in the field of healthcare, medicine, electronics [26]. the results of present study show the ability of AgNPs as antimicrobial agent.

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