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IN VIVO ACTIVITY OF GREEN ZINC OXIDE NANOPARTICLES AGAINST *Leishmania donovani* USING ALBINO MALE RATS

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ABSTRACT

The present study was designed to show the potential role of zinc oxide nanoparticles against the toxicity of *L. donovani*. The study were conducted on 15 adult male rats that arranged in three groups (5 rates for each), The control group that received normal saline, The second is the group with rats injected with *L. donovani* at dose rate of 1.2× 1 0 ^{6} cell/ 0.2ml . intraperitoneally, The third group is rats injected with *L. donovani* at a dose rate of 1.2× 1 0 ^{6} cell/ 0.2ml and treated with50ug/kg of ZnO-NPs for two weeks. The results show a high significant increased ($P < 0.05$) in levels of Malondialdehyde (MDA) and a high significant decreased ($P < 0.05$) in levels of GSH, catalase in the second group compared with the controls. The results of third group showed non-significant changes ($P < 0.05$) in all parameters compared with controls when using green zinc oxide nanoparticles. The results of the histopathological study of sections prepared from animals of the second group indicated a thickening wall of bronchiole in most regions with sever lymphocytes infiltration and damage in wall of alveoli. However , After treatment, the sections that prepared from the third group show a semi-normal structure of bronchiole, alveolar sac and alveoli. It has been was concluded that green zinc oxide nanoparticles have the true been potential role against the toxicity of *L. donovani* in adult male rats.

.INTRODUCTION

Leishmaniasis is one amongst the foremost numerous and sophisticated diseases caused by associate degree obligate intra cellular protozoan parasite happiness to the mastigophoran [1]. Kala azar may be a major public pathological state within the world, it's recognized as a vital public pathological state in Irak and also the most fatal kind, classic that is, referred to as Kala-azar or black fever [2-3]. L. donovani attacks the RES and also the symptoms area unit characterised by fever, hepato-splenomegaly, leukopenia, progressive weakness and leanness, which may lead to death if left untreated [4-5]. Recently, associate degree explosive strategic development has been habitually applied in novel inexperienced synthesis protocols that produces nanoscale biocompatible nanoparticles attributable to the chance of coming up with good bio-nanomaterials with specific biological functionalities. In recent times, non-toxic, biomimetic, surroundings friendly approaches are gaining additional importance thanks to their ability to create a good vary of biocompatible nanomaterials paves the thanks to best fitted for medical specialty applications[6-8]. Zinc oxide nanoparticles (ZnO-NP) encompasses a tremendous potential in biological applications like biological sensing, biological labeling, sequence delivery, drug delivery and nano-medicine [9-10] beside its bactericide, antifungal, acaricidal, pediculocidal, larvicidal and anti-diabetic activities [11-12]. Therefore the aim of study is to indicate the potential role of oxide nanoparticles against the toxicity of L. donovani.

MATERIALS AND METHODS

Source of Leishmania

The pure isolate of *L. donovani* was obtained and diagnosed at the Department of Biology – College of Education for Pure Science – University of Samaraa .

Plant extract

Saffron (*Crocus sativus L.*) flowers purchased from local markets were used. The aqueous solution of the saffron was obtained by grinding dried flowers in the form of fine powder by an electrolytic mill. 60 mg of the powder was added to 500 ml of distilled water and left at room temperature for 24 hours and the solution was filtered using a Buchner funnel and Wattman No.1 filter paper, The filtration solution dried using an oven at a temperature of 45 C°. after drying distilled water was used to prepare the concentrations under study [13].

Green synthesis of zinc oxide nanoparticles

20 ml of the plant extract was heated at 50 °C for 10 min and 50ml of 91 mM of zinc acetate solution (1 gm of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise to it under stirring. The reaction mixture became yellowish and cream colored precipitate of zinc hydroxide was formed. The reaction mixture was left for 30 min for complete reduction to zinc hydroxide. Then the precipitate was collected by centrifugation at 16000 rpm for 10 min at 4 °C. The precipitate was vacuum dried at 30 °C and the sample (PZN30) was stored by using [14].

Experimental design

Fifteen adult male rats were used in this study and then divided as follows (5 rates for each group):

1. control group: rats were received a normal diet only for two weeks and then killed.
2. Second group: rat injected intraperitoneal with *L. donovani* at dose 1.2× 1 0 ^{6} cell/ 0.2ml were, and then killed after infection.
3. Third group: rat injected intraperitoneal with *L. donovani* at dose 1.2× 1 0 ^{6} cell/ 0.2ml and treated with 50ug/kg ZnO-NPs for two weeks, and then killed.
4. Fourth group: rat treated with 50ug/kg ZnO-NPs for two weeks, and then killed.

2.5. Prepare of blood solution

Blood samples are collected from rat heart, under anesthesia, blood was put in test tubes. Then, tubes were centrifugation for 10 min to obtain serum that stored by deep freezing until used.

2.5. Serological tests

MDA (malonedialdehyied) was measured according to colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer [15]. Glutathione (GSH) level estimated by mixed 2.3 ml buffer with 0.2ml of the sample and then added 0.5ml of DTNB (5,5-dithio-bis-(2-nitrobenzoic acid). The mixture was analyzed by spectrophotometer [16].

Histopathological study

Lung biopsies were taken with 4mm punch and 2% xylocaine was used as an anesthetic. The biopsies were fixed in 10% formalin, routinely processed and embedded in paraffin sections which were stained with hematoxylin and eosin and examined under the microscope.

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) [17].

RESULTS

Oxidative stress (MDA) & antioxidant parameters (GSH and catalase)

The levels of MDA (2.67 ± 0.42), GSH (0.293 ± 0.019) and catalase (0.64 ± 0.03) in second group show high significant changes ($P < 0.05$) compared with controls (1.53 ± 0.19; 0.423 ± 0.034 and 1.22 ± 0.08 respectively). The levels of MDA (1.63 ± 0.27), GSH (0.415 ± 0.028) and

catalase (1.19 ± 0.06) in third group show non-significant changes ($P < 0.05$) compared with control rats as shown in Table (1).

Table (1): The levels of MDA, GSH and CAT in serum

Parameters / Groups	MDA (mmol/l)	GSH (mol/l)	Cata (mmol/l)
Control group	1.53 ± 0.19	0.423 ± 0.034	1.22 ± 0.08
Second group	2.67 ± 0.42 *	0.293 ± 0.019 *	0.64 ± 0.03 *
Third group	1.63 ± 0.27	0.415 ± 0.028	1.19 ± 0.06

Histopathological study

The sections that prepared with a control group that show normal in the normal structure of bronchiole, alveolar sac and alveoli as shown in figure (1). The cross section that prepared from second group that injected with *L. donovani* show thickening wall of bronchiole in most regions with sever lymphocyte infiltration and damage in wall of the alveoli as shown in figure (2). The sections that prepared from third group show a semi-normal structure of bronchiole, alveolar sac and alveoli as shown in figure (3).

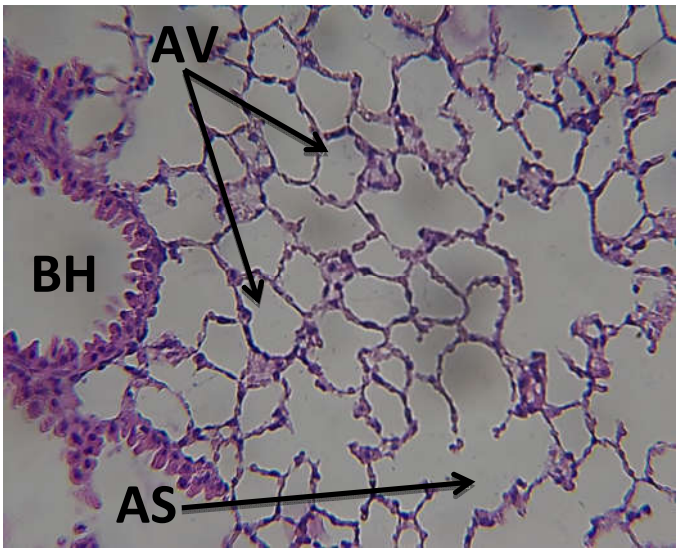

Figure (1): lung of third group show normal structure of bronchiole (BH), alveolar sac (AS) and alveoli (AV) H&E X400.

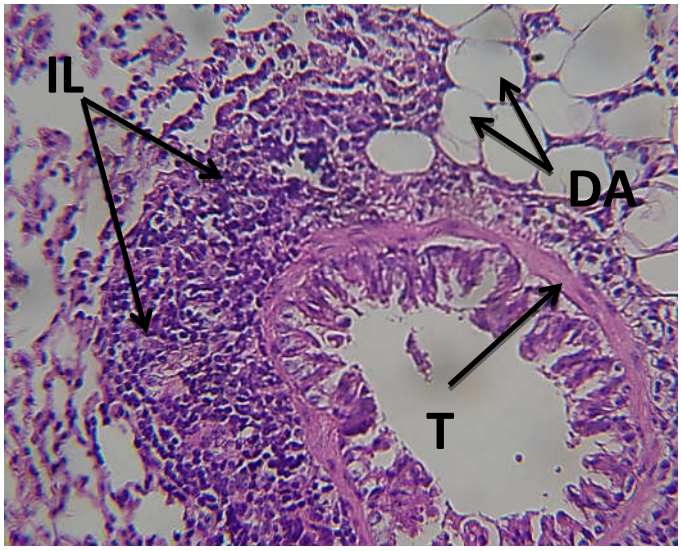

Figure (2): lung of second group show thickening wall of bronchiole (TW), lymphocytes infiltration (IL) and alveoli degeneration (DA) H&E X400.

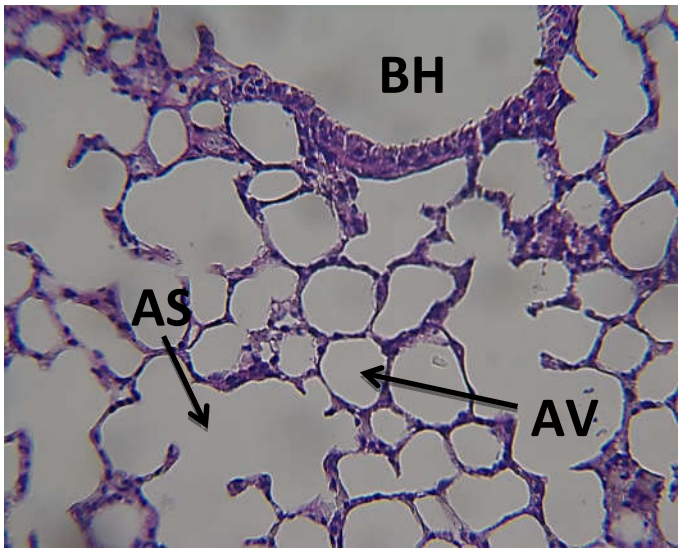

Figure (3): lung of third group show bronchiole (BH), alveolar sac (AS) and alveoli (AV) H&E X400.

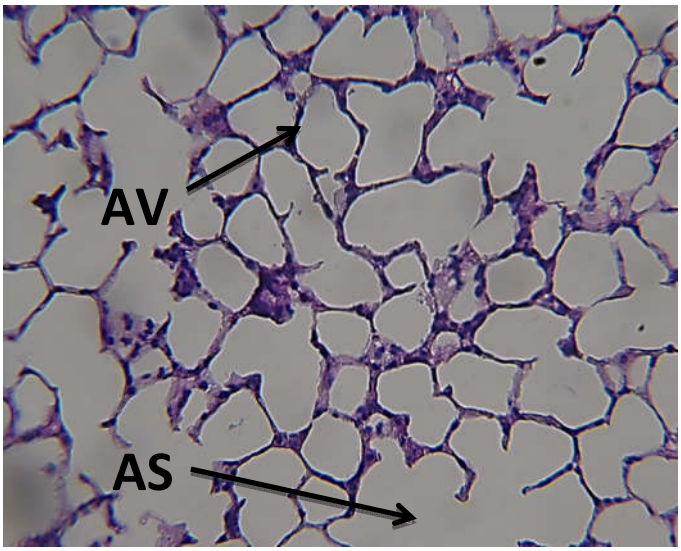

Figure (3): lung of fourth group show alveolar sac (AS) and alveoli (AV) H&E X400.

DISCUSSION

The results of ,present study show different significant changes in oxidative stress and antioxidant enzymes, with different lesions in lung tissue of ,rat that injected with *L. donovani*. The Same results were reported by Ali (2016) who referred that visceral Leishmaniasis that caused by *L. donovani* lead to increase the levels of MDA (4.60±2.15) and decrease the levels of catalase (0.75±0.58) compared to control group (1.35±0.31; 9.81±1.16 respectively) [18]. The numerous increase of MDA level within the gift study powerfully reflects associate in Nursing hyperbolic lipide peroxidation initiated by reaction of free radicals to unsaturated fatty acids in biological membranes. lipide peroxidation is created by oxidative stress ensuing from the over-production of ROS and element species (RNS) in, cutaneous Leishmaniasis [19]. Also, Also, in an alternative study according a decrease in enzyme activity and also the increase MDA levels in patient with Delhi boil are according. many inhibitor enzymes like SOD and CAT exist that convert ROS into harmless merchandise [20]. About, , the treatment with nanoparticles, compounds that found in plants extract, are bound to ions, and function as a complexing agent [21]. There are a number ,of, biologically active constituents in Saffron (Crocus sativus) ,including more than 34 components that are terpenes, terpene alcohols and their esters among which safranal is the main component. Non-volatile compounds, comprise crocins, crocetin, picrocrocin and flavonoids (quercetin and kaempferol) [22]. Many studies on medicinal properties of saffron have indicated that saffron, has a potent antioxidant activity which is ,mostly due to the presence of crocin as a unique carotenoid [23-24]. On the other hand, the role of AgNPs within the treatment was incontestable through its propeities as inhibitor and anti inflammatory. Whereas, David et al. according that Ag-NPs synthesized by American elder possess exceptional medicinal drug properties [25]. El-Rafie and Hamed according that the synthesized nanoparticles by Terminalia species have inhibitor activity thanks to the capped phenolic resin and flavonoid compounds and might be used against hurtful effects of free radicals and have powerfully medicinal drug activity [26].

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