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The Modulation of the Oxidative Stress Profile in Various Organs of *Trypanosoma congolense*-Infected Rats by Ellagic Acid

Mohammed A. Ibrahim[1*], Auwal Adamu[1], Funmilola E. Audu[1], Mukhtar A. Suleiman[1], Rapheal Aminu[1], Abubakar B. Aliyu[2], Maryam Danfulani[1], Emmanuel J. Bakura[1], Samuel N. Tsako[1] and Aminu Mohammed[1]

[1]*Department of Biochemistry,* [2]*Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria*

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Abstract

Ellagic acid has been previously found to possess trypanostatic effects and to alleviate some of organs' pathological complications, but it was not known whether these effects were mediated through an antioxidant-related mechanism or not. This work, therefore, investigates the effects of ellagic acid on lipid peroxidation and the antioxidants profile of *Trypanosoma congolense*-infected rats. The rats were infected with *T. congolense* and treated with ellagic acid (100 and 200 mg/kg body weight (BW)) and diminazine aceturate (3.5 mg/kg BW) for fourteen days; the remaining infected group was left untreated, while some animals were uninfected and untreated. The organs (liver, kidney, spleen, and heart) were harvested and homogenized, and malonyldialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase levels were measured. The MDA levels were significantly decreased ($P < 0.05$) across all organs in the ellagic acid-treated groups compared to the infected untreated group. There was a significant increase ($P < 0.05$) in the GSH levels in the group treated with 200 mg/kg BW of ellagic acid across all organs. However, treatment with ellagic acid did not significantly ($P > 0.05$) change the SOD level compared to the infected untreated group in the liver of rats, but an increase was observed in the kidney, spleen, and heart of the treated groups. The 100 mg/kg BW of ellagic acid increased the catalase levels ($P < 0.05$) in all organs except the kidney. This study concluded that ellagic acid boosted the endogenous antioxidant reserves and reduced lipid peroxidation.

KeyWords: *Trypanosoma congolense*, Oxidative stress, Antioxidant, Ellagic acid, Organ damage.

1. Introduction

Trypanosomiasis is a neglected tropical disease affecting humans and livestock production with tremendous impacts on the socio-economic systems of many under-developed countries (Welburn *et al.,* 2009; Samdi *et al.,* 2010; Lozano, 2012). It is caused by different trypanosome species but *Trypanosoma congolense* is considered to be the most prevalent and a major impediment to livestock production in Africa. This parasite is a strictly intravascular parasite and without conspicuous undulating membrane (Abubakar *et al.,* 2015).

Considerable evidence has demonstrated the roles of reactive oxygen species in the complications associated with the *T. congolense* infections (Umar *et al.,* 2009; Ibrahim *et al.,* 2016; Igbokwe *et al.,* 2018). These reactive oxygen species including free radicals are usually released by the trypanosome, and are known to target the membrane polyunsaturated fatty acids and proteins of the host cells leading to cellular damage and consequently affecting the performance of important tissues and organs such as the kidneys, liver, heart and brain of the infected animals (Umar *et al.,* 2009; Baldissera *et al.,* 2016; Igbokwe *et al.,* 2018). In fact, the development of organ damage as a result of the trypanosome-associated oxidative stress is important in the pathogenesis of the disease and is mainly responsible for the death of infected animals (Ibrahim *et al.,* 2013). Interestingly, animals are endowed with endogenous antioxidants such as glutathione, catalase and superoxide dismutase (SOD), which mop up the free radicals and protect the infected animals against the oxidative stress-related pathogenic features of the *T. congolense* infections (Umar *et al.,* 2009; Ibrahim *et al.,* 2016; Igbokwe *et al.,* 2018).

Recently, the search for chemotherapeutic agents against man and livestock diseases has exponentially increased (Odhiambo *et al.,* 2011). With respect to trypanosome infections, 264 medicinal plant species from seventy-nine plant families across the sub regions of Africa were reported to possess *in vitro*/*in vivo* antitrypanosomal activities after an extensive review (Ibrahim *et al.,* 2014). In drug discovery, the identification of bioactive compounds responsible for an observed activity is an important endeavour, but only forty-eight compounds were isolated and characterised, with certainty, from the above-mentioned 264 plants as the bioactive trypanocides. One of these compounds is ellagic acid; a phenolic acid found in plants and is produced from the hydrolysis of tannins such as geranin and ellagitannin (Seeram *et al.,* 2005). The *in vitro* antitrypanosomal activity of ellagic acid has been reported, and it was also demonstrated to be safe (Shuaibu *et al.,* 2008). In addition, its *in vivo* trypanosuppresive effects was recently reported and found to alleviate the trypanosome-associated aneamia

* Corresponding author e-mail: mauwalibrahim@gmail.com or maibrahim@abu.edu.ng.

and organ pathological alterations (Aminu *et al.*, 2017). However, it is not known whether the observed effects of ellagic acid on the organs of the *T. congolense*-infected animals were mediated, at least in part, via an antioxidative-dependent mechanism.

Based on the above observation coupled with the crucial role of oxidative stress to the pathogenesis of the *T. congolense* infection, the present study investigates the effects of ellagic acid on the *in vivo* antioxidant defence system of the liver, kidneys, spleen and heart of *T. congolense*-infected animals.

2. Materials and Methods

2.1. Chemicals and Reagents

The ellagic acid and dimethyl sulphoxide (DMSO) were procured from Sigma Chemical Company USA, through a local vendor (Bristol Scientific Company Limited, Lagos, Nigeria). Kem Light Laboratory limited, Mumbai, India, and Eagle Chemical Company Ltd, Ikeja, Nigeria, provided the thiobarbituric acid (TBA) and the diminazine aceturate respectively.

2.2. Experimental Animals and Trypanosome Parasites

The guidelines of the Good Laboratory Practice (GLP) and the rules and regulations of experimental animal ethics committee of ABUZ were followed in the study. Wistar rats weighing 170–220 g were procured from the animal house of the Department of Pharmacology and Therapeutics, at the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, (ABUZ) Nigeria. The animals had unlimited supply of commercial rat chow (ECWA Feeds, Jos, Nigeria) and water *ad libitum*. The *T. congolense* (savannah strain) was obtained from the National Institute of Trypanosomiasis and Onchocerciasis Research (NITOR), Kaduna, Nigeria. The parasites were passaged into the rats until a peak parasitemia was achieved. Then, the parasitized blood was collected and diluted in a physiologically cold saline before it was used for the infection of experimental animals.

2.3. Animal Grouping and Administration

Twenty-five rats were randomly allocated into five groups. Rats in four of the groups were each infected by an intraperitoneal injection of approximately 1×10^4 *T. congolense* per 100 g body weight (BW), while the rats in the remaining group were uninfected. Approximately, 10^6 trypanosomes/ml of blood was achieved on day eleven post-infection (pi) and the treatment commenced. Two groups of the infected rats were given oral treatment that is 100 (ITEA100) and 200 mg/kg BW (ITEA200) of ellagic acid on a daily basis, whereas another group was treated with 3.5 mg/kg BW of diminazine aceturate (ITDA) and the remaining group of infected rats was left untreated (IC). On the other hand, the uninfected group of rats was also left untreated (NC).

2.4. Collection of Organs and Tissue Processing

The experiment was terminated on day twenty-four pi and the animals were euthanized by chloroform anaesthesia. The organs (liver, kidney, spleen and heart) were also collected from each animal, and were washed with normal saline to get rid of adhered tissues and wiped with filter paper. The organ fragments were homogenized (1:10 w/v) using a homogenizer, and centrifuged at 800 xg for ten minutes at 4˚C. The supernatant was further centrifuged at 10, 000 xg for twenty minutes, and the newly-recovered supernatant was also collected in another microtube and stored at −20 °C for the analysis of antioxidative parameters.

2.5. Analysis

The reactive substances of thiobarbituric acid, expressed as malondialdehyde (MDA) equivalent, were assessed to determine the extent of lipid peroxidation in the organs (Fraga *et al.*, 1988). The reduced glutathione (GSH) level was determined using the DTNB method (Ellman, 1959). The catalase activity was monitored as described by Aebi (1984), while the superoxide dismutase (SOD) activity was measured based on its ability to inhibit the auto oxidation of epinephrine as described by Misra and Fridovich (1972).

2.6. Statistical Analysis

All data were shown as mean ± standard deviation of five animals. Data were analyzed by using a statistical software package (SPSS for Windows, version 20, NY, USA) using Tukey's-HSD multiple range post-hoc test. Values with $P<0.05$ were considered significantly different between each other.

3. Results

The data of lipid peroxidation were expressed as the malondialdehyde (MDA) concentration in the organs (liver, kidney, spleen and heart) of all the groups and are presented in Figure 1. The *T. congolense* infection significantly ($P < 0.05$) increased the MDA levels across all organs compared to the NC group. However, the oral administration of ellagic acid significantly ($P < 0.05$) reduced the MDA levels in the kidney, spleen and heart of the infected animals, but not so different from the ITDA group. Meanwhile, in the liver, a dose of only 100 mg/kg BW of ellagic acid (ITEA100) reduced the MDA levels significantly ($P < 0.05$). The results of the reduced glutathione (GSH) concentrations revealed significantly ($P < 0.05$) decreased levels across all organs in the IC group compared to the NC group. However, the treatment with ellagic acid significantly ($P < 0.05$) increased the levels of GSH in all the organs, and the ITEA200 group showed a significantly higher ($P < 0.05$) GSH level than the ITEA100 group (Figure 2)

Figure 3 presents the activity of superoxide dismutase (SOD) of the serum and organs of all the experimental groups. It was observed that the activity of SOD of the IC group was significantly ($P < 0.05$) decreased in the liver and heart, while there was an insignificant ($P > 0.05$) reduction in the kidneys and spleen. After the administration of ellagic acid, the activity of SOD was increased, but not significantly ($P < 0.05$), in the liver and heart of the ITEA100 and ITEA200 groups compared to the IC group (Figure 3). Moreover, the activity of SOD in the kidneys and spleen of the ITEA groups was increased, but not significantly ($P > 0.05$) compared to the NC and IC groups (Figure 3). The catalase activities in the organs of all the experimental groups are presented in Figure 4. Interestingly, the activity of the catalase did not differ significantly ($P > 0.05$) across all the organs when compared to the NC group. However, there was a significant ($P < 0.05$) increase in the activity of catalase in the ITEA100 group in the kidneys, spleen, and heart.

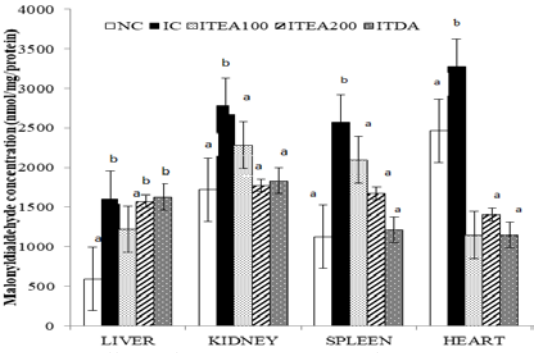

Figure 1. Effects of oral administration of ellagic acid on lipid peroxidation (MDA) in the organs of *T. congolense* infected rats. Data was presented as mean ± SD (n=5). [a-c]Values with different letter over the bars for a given sample are significantly different from each other (Tukey's multiple range post-hoc test, $P < 0.05$). NC, Normal Control; IC, infected Control; ITEA100 and ITEA200 are infected groups that were treated with 100 and 200 mg/kg BW of ellagic acid respectively while ITDA is an infected group treated with 3.5 mg/kg BW of diminazine aceturate.

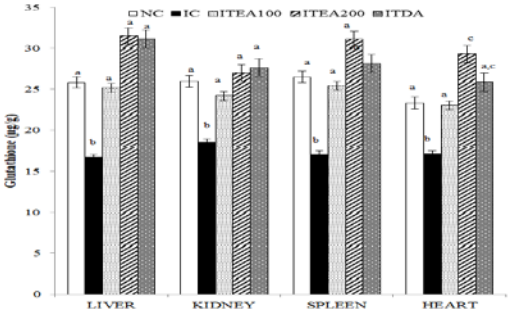

Figure 2. Effects of oral administration of ellagic acid on glutathione (GSH) in the organs of *T. congolense* infected rats. Data was presented as mean ± SD (n=5). [a-c]Values with different letter over the bars for a given sample are significantly different from each other (Tukey's multiple range post-hoc test, $P < 0.05$). NC, Normal Control; IC, infected Control; ITEA100 and ITEA200 are infected groups that were treated with 100 and 200 mg/kg BW of ellagic acid respectively while ITDA is an infected group treated with 3.5 mg/kg BW of diminazine aceturate.

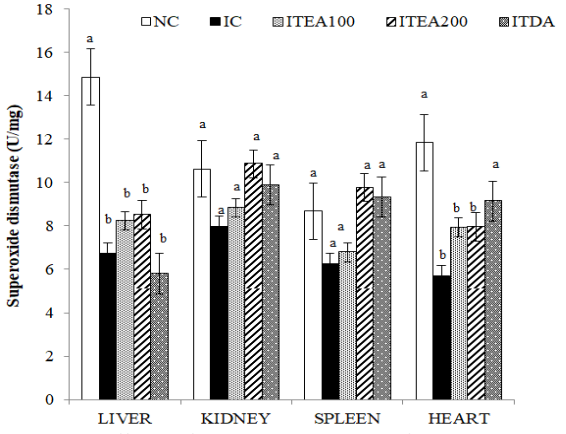

Figure 3. Effects of oral administration of ellagic acid on superoxide dismutase (SOD) activities in the organs of *T. congolense* infected rats. Data was presented as mean ± SD (n=5). [a-c]Values with different letter over the bars for a given sample are significantly different from each other (Tukey's multiple range post-hoc test, $P < 0.05$). NC, Normal Control; IC, infected Control; ITEA100 and ITEA200 are infected groups that were treated with 100 and 200 mg/kg BW of ellagic acid respectively while ITDA is an infected group treated with 3.5 mg/kg BW of diminazine aceturate.

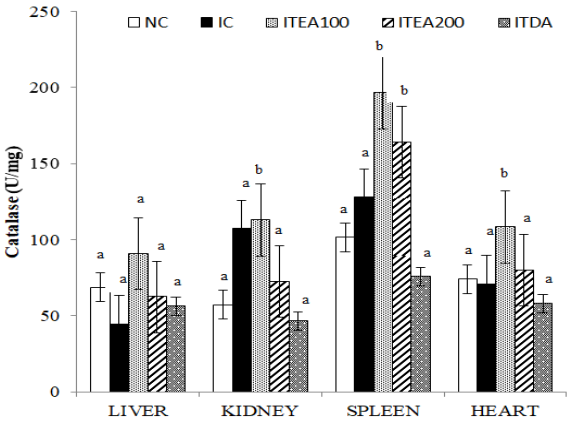

Figure 4. Effects of oral administration of ellagic acid on catalase activity in the organs of *T. congolense* infected rats. Data was presented as mean ± SD (n=5). [a-c]Values with different letter over the bars for a given sample are significantly different from each other (Tukey's multiple range post-hoc test, $P < 0.05$). NC, Normal Control; IC, infected Control; ITEA100 and ITEA200 are infected groups that were treated with 100 and 200 mg/kg BW of ellagic acid respectively while ITDA is an infected group treated with 3.5 mg/kg BW of diminazine aceturate.

4. Discussion

The pathogenesis of *T. congolense* infection is related to the induction of oxidative stress as a key biological player as previously reported in trypanosome infections (Gupta *et al.*, 2009; Ibrahim *et al.*, 2016). Hence, some antitrypanosomal candidates mediate beneficial effects via an antioxidant-dependent mechanism in the infected animals. Therefore, it was interesting to note that the ellagic-acid treatments significantly reduced the MDA level in the liver and kidneys of the infected treated rats and also improved the antioxidant levels suggesting that the degree of the *T. congolense*-related oxidative stress on these organs was mitigated. Perhaps, this might explain, at least in part, how the observed amelioration of *T. congolense*-induced liver and kidney damages by the compound (Aminu *et al.*, 2017) was mediated through the alleviation of oxidative stress in the animals.

A typical mechanism for red-blood cell elimination during the *T. congolense* infection is erythrophagocytosis in the spleen and liver (Murray and Dexter, 1988) which eventually alters the integrity of these organs and enhances the production of free radicals. On the other hand, El-Deeb and Elmoslemany (2015) and Baldissera *et al.* (2016) reported oxidative stress in the heart of animals having a trypanosome infection and also suggested correlation between serum levels of biomarkers of cardiac injury and oxidative stress. Interestingly, ellagic acid significantly improved oxidative stress in the spleen and heart of the infected treated rats. Therefore, it is plausible that ellagic acid prevented the *T. congolense*-induced damage to these organs through the modulation of the trypanosome-related oxidative stress suggesting an antioxidant-dependent mechanism.

Overall, the protective efficacy of ellagic acid to avert lipid peroxidation might be attributed to its ability to interact with free radicals generated by *T. congolense*. It is also possible that the observation is linked to the observed trypanosuppressive effects of the compound (Aminu *et al.*,

2017) which consequently reduced the amount of free radicals generated by the parasites.

In summary, ellagic acid mitigated the trypanosome-induced oxidative stress and boosted the reserves of endogenous antioxidant systems in the *T. congolense*-infected animals, which makes it a therapeutically beneficial agent against diseases affecting livestock.

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Compliance with Ethical Standards Funding:

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Conflicts of Interest

There is no conflict of interest in this study. Also, all the authors have declared that they have no conflict of interest.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed Consent

This article does not contain any studies with animal performed by any of the authors.

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