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EFFECT OF *Abrus precatorious* METHANOL EXTRACT ON ANTIOXIDANT ENZYMES IN *Trypanosoma brucei bruce* INFECTED MICE

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ABSTRACT
Trypanosomiasis remains a group of diseases that are on the increase and brought about by a protozoan parasite from the species of Trypanosoma and has a lot of effects on the health and economic status of the endemic populace. The causation of trypanosomiasis is associated with high production of Reactive Oxygen Species (ROS) and free radicals which are toxic to the cell structures. The study was performed using an *in vivo* to investigate the impact of *Abrus precatorious* methanol extract on antioxidant enzymes in *Trypanosoma brucei* (T.b) infected mice. Single dose toxicity studies were performed according to procedure outlined by Lorke. Infected mice were administered with 200, 400 and 600mg/kg BW of the crude extracts for two consecutive weeks. To assess efficacy and antioxidant ability, three other groups; normal control, untreated control and Diaminazine aceturate treated (standard control) were used. The immediate toxicity experiments revealed that *Abrus precatorious* extract remained safe at a level of 5000 mg/kg BW. A significant increase ($p < 0.5$) in the serum and liver SOD, GPx, and Catalase activity of the *Abrus precatorius* methanol extract therapy groups were observed compared to the untreated rats. Current study showed that the methanol extract from *Abrus precatorious* could elevate the life span of erythrocytes since the overwhelming erythrophagocytosis produces free radicals in trypanosome infection and subsequently improving antioxidant enzymes.
Keywords: Antioxidant enzymes Abrus precatorious and Trypanosoma brucei brucei

1.0 INTRODUCTION
Trypanosomiasis is one of the Neglected Tropical Disease (NTDs) which affects over 60 million people and livestock in sub-Saharan Africa (FAO, 2024 as cited in Papagni et al., 2023). The disease is produced from a protozoan parasite belonging to the Trypanosoma genus; the tsetse fly transmits the disease to both man and animals (Balasegaram et al., 2008). Drugs are the only available management and chemotherapeutic options for the treatment of trypanosomiasis. However, most of the available drugs have serious limitations, which include high cost, long-course of parenteral administration, adverse effects and emergence of drug-resistant trypanosome strains (Maikai *et al.,*2008, Simarro *et al*., 2009). Trypanosomiasis is fatal if not properly treated due to the progressive nature of the diseases especially at the neurological (second) stage when the parasite crosses the blood -brain barrier (CDC, 2020; Rogers *et al*., 2017). Recent investigations have shown that increased oxidative stress is one of the main reasons of morbidity and death in trypanosomiasis.

The trypanosome infection and consequent oxidative stress induce leucopenia, anemia, thrombocytopenia, tissue inflammation and injury, splenomegaly and cachexia (Bezie et al., 2014). Several literature detailing the antioxidant activities paid much attention to total antioxidant activities of plant extracts (Madaki et al., 2019), while very few provided insight into the effect of the extracts on oxidative stress biomarkers in diseases, for instance, trypanosomiasis. However, only few of such studies pay attention to the level of damage biomarker of oxidative stress (Abubakar and Dabo, 2023) in the context of the progressive stage of the disease.

Antiparasitic activities have been reported for several Nigerian plants (Ogbadoyi *et al*. 2011; Madaki *et al*. 2016; Santhosam *et al*. 2023; Abubakar & Dabo, 2023). *Abrus precatorious* belongs to the family *Fabaceae*, it is a perennial shrub, it flowers are purple pink and clustered, the fruits are pod bearing characteristic red seeds with black spot (Tabsum *et al*., 2016). In Nigeria, *Abrus precatorius* is employed in order to treat malaria, typhoid fever, hepatitis and arthritis and in the management of respiratory tract infections including asthma (Georgewill and Georgewill, 2009; Taur and Patil, 2011; Santhosam et al., 2023). We earlier documented the *in vitro* antioxidant activity of *Abrus precatorius* in our previous study (Madaki et al., 2019). Thus, it was necessary to determine whether plant extract can effectively and feasibly change the antioxidant enzymes. The present investigations observed the impact of methanol leaf extract of *Abrus precatorius* on antioxidant enzymes in *Trypanosoma brucei* induced mice.

2.0 MATERIALS AND METHODS

Materials

Reagent and chemicals

The organic solvent methanol used in the extraction of the plant material was item of Sigma Chemical CO St. Louis M.O (USA). Completely biochemical assay kits utilized in this study had been purchased from Randox Laboratories Ltd in United Kingdom and from Agape diagnostics in Switzerland. Every chemical used in the work was of laboratory- analytical grade quality.

Experimental animals

Albino mice used for screening were bought from the National Institute of Trypanosomiasis and Onchocerciasis, (NITR) Kaduna, Nigeria. Wistar rats (35.65± 3.89 g) were kept in the Animal Holding Unit, Biochemistry Department of Federal University of Technology, Minna, Nigeria, to acclimatized. All experiments involving the animals were conducted in compliance with the ethical conduct (Assigned Number: 000082) of Federal University of Technology, Minna, Nigeria.

Plant Materials

Abrus precatorius was screened for antitrypanosomal activities. The plant sample were gathered from Minna, Niger state, during May and the month of August 2019. *Abrus precatorius* was deposited at the herbarium of University of Ilorin for identification and voucher numbers was allocated as UILH/001/2019/574.

Trypanosoma brucei brucei

The parasite used in this study *Trypanosoma brucei brucei* was from National Institute of Trypanosomiasis and Onchocerciasis Studies, Kaduna State, Nigeria; preserved at the Department's laboratory through serial passage within mice.

METHODS

Plant Samples Preparation

From fresh plant of *Abrus precatorius*, one (1) kg of the leaf was cleaned under running water and allowed to dry at the laboratory temperature. Grinding and sizing of the dried plant sample was done using an electric blending machine, when necessary, the sample was further milled into powder form. The powdered sample was kept in clean polythene bags for later use depending on the need to use them Kabiru et al. 2013.

Preparation of crude extracts

One hundred grams (100 g) of the pounded dried Abrus precatorius plant was measured as well as extracted with 600 ml of methanol using cold the maceration procedure as reported by Kolle et al. 2023, via minor variations.

Test organism Preparation

The parasite was kept circulating within a laboratory situation through passage in mice until needed. In passaging, about 1x10-3 parasites were inoculated intraperitoneally using blood from the infected animals between 0.1 and 0.2 ml in Phosphate Buffered Saline (PBS) solution as described by (Kabiru et al., 2013).

In-vivo antioxidant research

Collection and preparation of serum and liver sample

Collection of blood sample was done based on the procedure reported by Shittu *et al*., 2023. Total GPx (glutathione peroxidase) concentration within tissues has been measured using the Yusuf et al., 2022. Super oxide dismutase is an enzyme that catalyses the process of dismutation of superoxide formed out of oxygen in the tissues. This was done by the method of Yusuf *et al*., 2022. Catalase in serum was assayed by the modified method according to Sani *et al.,* 2022. Total protein in the serum was evaluated through a complete protein reagent (Randox Laboratories, UK) based on the Biuret technique, as described by Onoja et al., 2022.

Evaluation of data

Every experiment was performed in triplicates and data was depicted as Mean ± SEM. Multiple comparisons between the groups were performed using unpaired Student't-test or ANOVA through Graph pad prism4.0 software. The level of significance used for the study was $P < 0.05$.

RESULTS

Total liver and serum proteins

Infected untreated mice had substantially lower serum and liver protein concentrations ($p<0.05$) compared to normal controls figure 4. Methanol extract of *Abrus precatorius* significantly ($p<0.05$) raised blood as well as liver overall protein levels in infected mice relative to the control group without therapy. Diaminazene aceturate had the highest proteins in the liver sample of experimental groups.

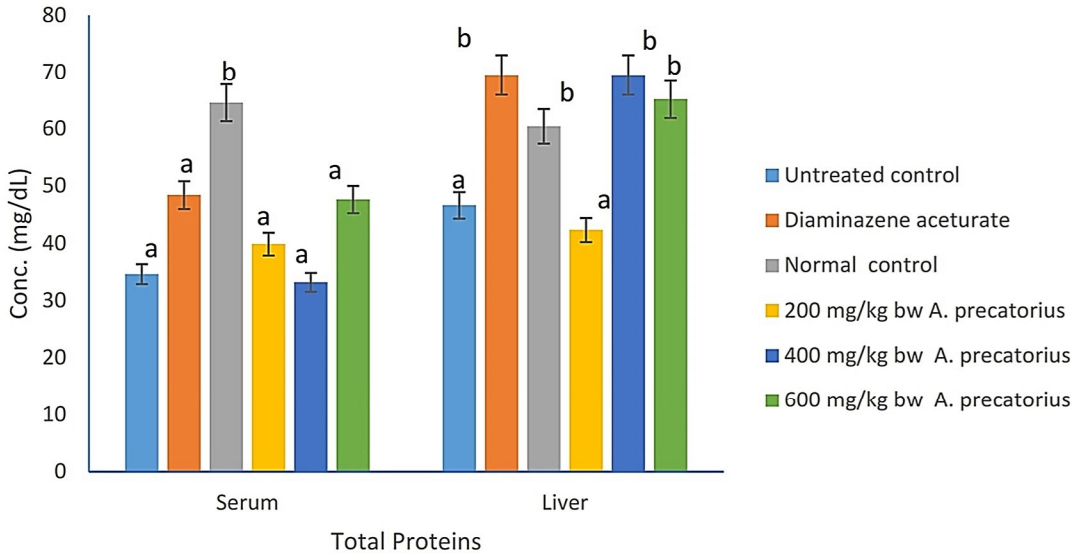

Figure 4: The Effect of *Abrus precatorius* Methanol Extract on Serum and Liver Total Proteins Concentrations in *Trypanosoma brucei brucei* Infected Mice

The results shown represent the average ± SEM from three measurements. Bars with different superscripts indicate significant differences ($p < 0.05$).

Glutathione peroxidase activity in Serum and liver

Uninfected and treated mice had significantly greater ($p<0.05$) GPx actions in the liver and lesser ($p<0.05$) GPx activities in the serum compared to normal control and treatment groups. Figure 5 shows that mice injected with 600 mg/kg had the greatest serum glutathione peroxidase activity when compared to the control as well as the other experimental groups of animals. Infected untreated mice had significantly lower liver GPx production ($p<0.05$) compared to the normal control as well as treatment groups.

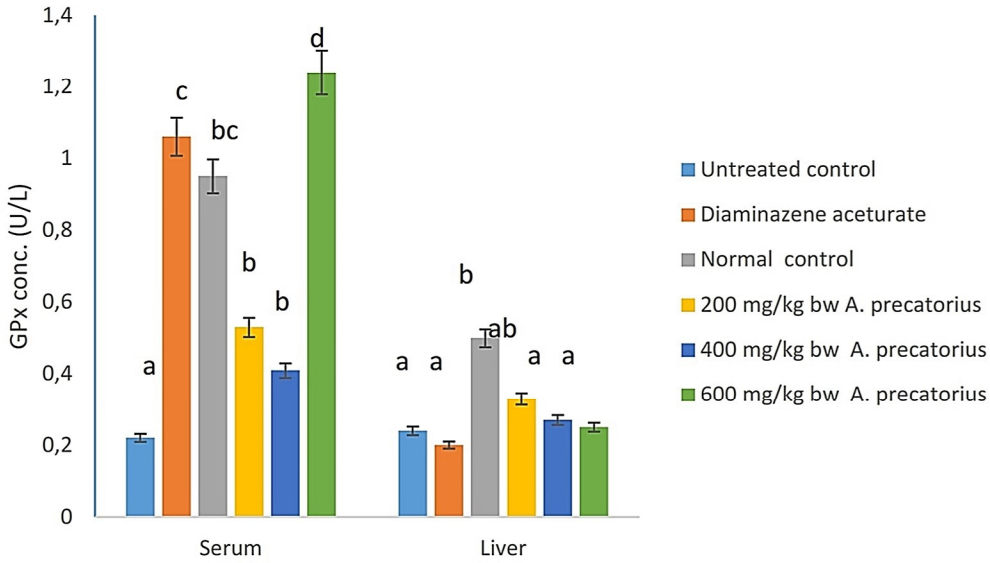

Figure 5: **The Effect of *Abrus precatorius* Methanol Extract on Serum and Liver Glutathione peroxidase activity in *Trypanosoma brucei brucei* Infected Mice**
The results shown represent the average ± SEM from three measurements. Bars with different superscripts indicate significant differences ($p < 0.05$).

Superoxide dismutase activity of serum and liver
Compared to normal control infected untreated mice presented significantly ($p<0.05$) low serum and liver activity of superoxide dismutase (SOD) Figure 6. Administered methanol extract of *Abrus precatorius* to infected mice enhanced serum and liver superoxide dismutase (SOD) in the treated group than the untreated group.

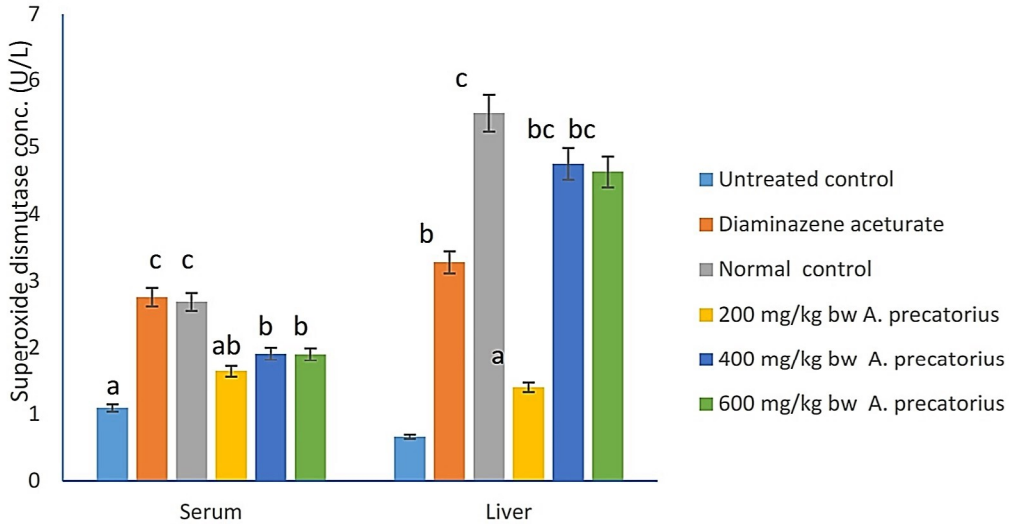

Figure 6: The Effect of *Abru precatorius* Methanol on Serum and Liver Superoxide Dismutase Activity in *Trypanosoma brucei brucei* infected mice
The results shown represent the average ± SEM from three measurements. Bars with different superscripts indicate significant differences ($p < 0.05$).

Catalase activities of Serum and liver

The infected untreated mice exhibited significantly ($p<0.05$) lowered catalase activities in liver as compared to normal control, as well as other groups as indicated in (Figure 7). Treatment of infected mice with methanol extract of *Abrus precatorius* significantly increases the liver catalase activities when compared with the untreated control. Mice treated with 600 mg/kg bw recorded the highest liver catalase activities compared with control and other experimental group

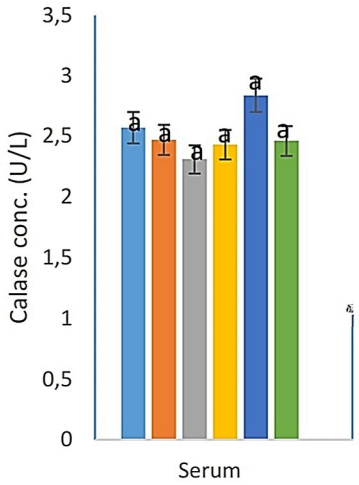

Figure 7: The effect of *Abru precatorius* Methanol Extract on Serum and Liver Catalase Activity in *Trypanosoma brucei brucei* infected mice

The results shown represent the average ± SEM from three measurements. Bars with different superscripts indicate significant differences ($p < 0.05$).

DISCUSSION

Oxidative strain and the modification from intrinsic antioxidant enzymes as factors in trypanosomiasis development (*Akpa et al*., 2021). The oxidative stress marker and parameters observed in the present study include, Liver and Serum; Total Protein- TP, catalase- CAT, glutathione peroxidase- GPX as well as superoxide dismutase- SOD. SOD is known to be a first line enzymatic defence enzyme that reduces the rate of dismutation of superoxide anion to molecular hydrogen and water. CAT and GPX scavenge the product of SOD activities H_2 O_2 to generate harmless molecular oxygen and water molecules (Ighodaro and Akintoye, 2017).

A number of researchers have opined that trypanosomiasis causes oxidative stress and reduces antioxidants enzymes while supplemented antioxidants strengthen the endogenous antioxidant system (Akanji et al., 2009). In the present work, the reduction of SOD, catalase and GPx, both in the serum and liver tissues of animals infected with *T. brucei* served as the indices of oxidative stress were observed. This result pointed to the fact that; the infection resulted to low levels of major antioxidant defence mechanism of the animals. It has been evidenced that trypanosomes infection leads to high levels of ROS and free radicals that are toxic to the cellular framework, especially; proteins and lipids (Mishra et al., 2017). This could be linked with erythrophagocytosis when trypanosome feeds on erythrocytes in animals.

Furthermore, there is direct damage to the red cells, acceleration of the rate of their removal from circulation by haemolytic factors released by the dying trypanosomes, immune complexes attached to Red Blood Cell (RBC) in addition to fever and mechanical damage to RBCs by the trypanosomes (Taylor and Authie, 2004).

Similarly, the liver total proteins concentrations were reduced significantly ($p<0.05$) in infected untreated mice than normal control. Enhancement of the total protein levels in serum and liver of *Abrus precatorius* methanol extract treatment groups as well as diaminazene aceturate treated group were observed. Similar observation of lowered serum total protein has also been described in the *T brucei* infected boars (Otesile et al., 1991). However, free radicals are neutralized through enzymatic activities of SOD, catalase or Glutathione peroxidase or non-enzymatically by undergoing chemical reactions with polyphenols, curcumin and β carotene and several other compounds (Fracasso *et al*., 2021). Other authors have mentioned the antioxidant perspectives of bio active compounds (Agbaji *et al*., 2013; *Gul et al*., 2013; Jain *et al.,* 2015); conversely, our earlier in vitro antioxidant investigations yielded high concentrations of saponin, flavonoids, tannins and phenols in *Abrus precatorius* methanol extract (Madaki *et al*., 2019).

The antioxidant potential of the extract against the free radicals formed during *T. brucei* was exhibited by the increase in the antioxidant defence system in the sera and liver of the animals. This is in line with a previous experiment which show raise in Antioxidant enzymes with treatment of *Azadirachta indica* against trypanosome - induced oxidative stress in dogs (Omobowale *et al*., 2015). It was established in this study that CAT, SOD, GPX activities were inhibited with *T. brucei* infection and a dose-dependent increase was observed with the highest at 600 mg/kg *A. precatorious.* The observed activities of the extract against free radicals could therefore be attributed to the composition of phytoconstituents. This significant decrease could possibly be attributable to the mobilization of defensive enzymes (which are known proteins) to counter the effect of trypanosome induced oxidative stress which were which in turn were managed by *A. precatorious.*

CONCLUSION

The results showed that methanol extract of A. precatorious possesses antioxidant activity, as evidenced by the extract's capacity to improve the actions of SOD, CAT, GPx, as well as TP in infected mice. Therefore *A. precatorious* methanol extract has improved antioxidant capacity of mice infected with *Trypanosoma brucei* by its virtue of bioactive components.

RECOMMENDATION

Further studies on bio - guided separation and characterization of *A. precatorious* is necessary for discovering the bioactive components responsible for the antioxidant activity.

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