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ACTIVITY OF HUMAN PLASMA PROTEINS ON TRYPANOSOMIASIS

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ABSTRACT
It is the first one had been conducted in Egypt to evaluate the antitrypanosomal activity of human plasma proteins compared with diminazene aceturate (DA) using Albino rats as a biological model. It was also carried out to assess the behavior, hematological, biochemical, and histopathological changes that may be associated with the administration of plasmas of four blood groups (O+, AB+, B+, A+) at a dose of 0.5 ml/200 gm. b. wt., two times along the experiment, for a period of 42 days against *Trypanosoma evansi* infection. The in vitro test for antitrypanosomal activity revealed that all the tested plasmas cause the disappearance of the Trypanosoma parasite at a very low concentration (0.001%). Results also revealed that treated groups with the plasma proteins of O+ and B+ human blood groups exhibited the highest in vitro and in vivo antitrypanosomal effect against *T. evansi* followed by A+ and AB+ groups, and could be useful in the management of trypanosomiasis. Histopathological examination evidenced and confirmed that O+ treated animals followed by B+ did not suffer from abnormalities in contrast to A+ and AB+ treated animals.

KEYWORDS: Trypanosoma evansi, Plasma proteins, Hematology, Biochemical analysis, Histopathology, Egypt.

INTRODUCTION
Trypanosoma evansi is the most pathogenic trypanosome to most domestic and many wild mammals, causing Surra disease that varies according to the virulence of the strain of trypanosome, the species of the host, unspecific factors affecting the animal such as other infections and general stress, and the local epizootiological conditions.[1,2] Trypanocidal drugs constitute the principal method of control, as the development of vaccines, immunotherapy and the herbal preparations against trypanosomiasis is still in progress.[3,4] The existing drugs as veterinary trypanocides: isometamidium chloride, diminazene aceturate, quinapyramine and cymelarsan treatments of trypanosomiasis are challenged with problems comprising drug resistance, toxicity and expensive/limited drugs.[5,6] Thus, there is an urgent need for new antitrypanosomal drugs that are more effective and safer.

Humans were considered refractory to infection by *T. evansi*, but a case of human infection was reported in an Indian farmer in 2005.[7] After that, about 22% of the population in an Indian village were positive for the parasite using blood smears, serology and molecular techniques.[8] This highlighted how can *T. evansi* infect a human, especially humans have innate immunity against *T. brucei*. This immunity against *T. b. brucei* is due to the trypanolytic activity of a human-specific apolipoprotein bound to high-density lipoproteins, termed apolipoprotein L-I (APOL1). Researchers attributed this condition due to the presence of apolipoprotein L-1 (APOL1) in human blood, and this protein would have a trypanocidal factor since it has the ability to lyse *T. brucei* (Pérez-Morga et al., 2005).[9] Later, they were found an APOL1 deficiency in the blood of these Indian people, which could have contributed to the development of the disease.[10]

APOL1 is one of the 2 primate-specific proteins, however, TLF-1 also contains haptoglobin (Hp)-related protein (Hpr), as well as apolipoprotein A-1 (APOA1) which are both, directly and indirectly, related to the toxicity to trypanosomes. Another related protein complex, TLF-2, although poorly characterized, is also likely to contribute to trypanosome killing. Recently, a

research group found that mice infected with *T. evansi* when treated with human plasma and blood can eliminate the parasite from circulation.[11] The objective of present study was to determine the activity of human plasma proteins against *T. evansi* experimental infection, and its effect on clinical parameters.

MATERIALS AND METHODS

Experimental animals

Adult female albino Wistar rats, aged 16 weeks, with body weights of 150–200 g, were obtained from the National Research Center, Giza, Egypt. The animals were housed in small groups (n=6 each), inside propylene cages. The temperature in the experimental animal room was maintained at 25±2°C with 12 h dark: light cycle and 70±10% humidity. The animals were provided with commercial pelleted rodent food and drinking water ad libitum. The animals were allowed to acclimatize to the laboratory conditions for 10 days prior to the start of the study.

Treatment

The experimental animals were divided into seven groups of ten adult males each. Groups from 1 to 6 were infected with 0.1 mL of blood from mice containing 3 x 10^5 trypanosomes. The *T. evansi* strain was originally isolated from a naturally infected camel at El-Bassatein abattoir, Cairo, Egypt[4] and maintained by passages through mice. At a 5-day post-infection, after the parasites were detected in the bloodstream, animals of the groups from 1 to 4 were intraperitoneally administered with human plasma from four adult females clinically healthy with different blood groups (O+, AB+, B+, A+) at a dose of 0.5 ml/200 gm. b. wt., two times along the experiment, for a period of 42 days. For comparison, three encoded parallel groups from 5 to 7 were used: one group containing only *T. evansi* and the second group was given intraperitoneally diminazene aceturate® as reference values, whereas the third group maintained an uninfected untreated control. On the seven-day post-infection and after two days of plasma administrations, the parasitaemia level of rats in the control and plasma groups were checked three times a week by wet blood film prepared from tail blood at x40 magnification. Clinical signs including weight loss, edema of the face, prepatent period, longevity and animal mortality were also observed. The number of parasites seen per field under the microscope was counted and treatment efficacy was determined by the number of rats that did not show clinical signs post-treatment against *T. evansi* infection.

Hematological, biochemical and histopathological changes

Blood was collected once a week along the experiment from each group by heart puncture in two parts with and without heparin for haematological and biochemical estimation, for a period of 42 days. All sera samples were stored at 4°C until used. The hematology analysis was done manually whereas, the biochemical analysis was done with fully automated analyzed using commercial kits (Spectrum, Egypt) according to manufacturer's instructions. Suitable pieces of liver, kidney, lung, spleen, brain and heart were removed, washed in saline and fixed in Bouin's fluid for histopathological examinations as the method described by[12] The sections were stained with Haematoxylin and Eosin (H & E) stains[13], observed under the microscope, and lesions were recorded.

Statistical analysis

Data obtained were expressed as mean ±standard error of the mean. It was analyzed using SPSS version 20.0 (IBM SPSS Statics 20, USA). Significant differences in measurement traits were analyzed using one-way analysis of variance (ANOVA) and the post-hoc test was applied for multiple comparisons. Values of $P< 0.05$ and $P< 0.001$ were regarded as statistically significant and highly significant, respectively.

RESULTS AND DISCUSSION

Screening for antitrypanosomal properties

The in vitro test for antitrypanosomal activity revealed that all the tested plasma proteins at a very low concentration (0.001%) cause disappearance of Trypanosoma. Regarding safe dose and acute toxicity, the healthy rats were injected with serial of doses (0.1- 3 ml) to evaluate their safety for the rats showed that all of them are safe. According to[11], 1 ml of each plasma protein was selected for processing in vivo study.

Plasma proteins administration (in vivo experiment)

The in vivo studies showed that all *T. evansi* experimentally infected groups gave parasitaemia of approximately 10^3 parasites/ml with no significant differences between infected groups at day-3 post-infection (day 0 for treatment). DA and plasma proteins showed parasite clearance at 1st -day post-treatment in compared with T group. The second treatments were administrated for all groups in day-14 post-treatment. At 24 hr. after the second treatment (15th -day post-treatment), parasites were disappeared from DA, O+, B+, A+ and AB+ groups compared to T group. There was no a recrudescence of parasitaemia at day 21 post-treatment of the infected animals in DA, O+ and B+ groups, while the parasite reappeared in animals of A+ and AB+ groups. Nevertheless, comparison of parasitaemia with the animals of T revealed that the treatment with plasma protein in A+ and AB+ groups were kept parasitaemia at low level on day-21 post-treatment. At day-28 post-treatment, there was recrudescence of parasitaemia of the infected animals treated with DA drug. Whereas, the animals treated with plasma protein in O+ and B+ groups still showed parasite clearance in the *T. evansi* infected rats at 28th-day post-treatment. The parasite reappeared in animals of all treated groups at 35th-day post-treatment with the exception of O+ group where the animals of this group still clear from the parasite. At day-42 post-treatment, *T. evansi* detected in all treated groups and the lowest level reported in case of O+ group animals.

Comparison among all treated groups indicated that the plasma protein (O+ and B+ groups) exhibited the highest antitrypanosomal effect against *T. evansi* followed by A+ and AB+ groups. However, the Neg. group (not infected not treated) was healthy until the end of the experiment.

In the present study, the mean longevity of T group rats was of 25±4.95 days, while it was 42±0 days of the Neg. group and no rats from Neg., O+ and B+ groups died within 42 days. The treatments with plasma proteins have significantly increased the longevity of rats compared to the non-treated group (Table 1). This in agrees with the results of two recent studies by[11,14] who investigated the susceptibility of *T. evansi* to therapy using human blood and plasma in experimentally infected mice and rats, respectively. On the other hand, *T. evansi* infected rats in T group showed 83.33% mortality percentage during 42 days post-infection (Table 1). However, after the recrudescence, the animals may die due to the high level of parasitaemia.[15]

The explanation for the increased longevity and elimination of the circulating parasite 24 h after therapy with the plasma protein in the present results may due to the passive immunity resulting from APOL1 and/or haptoglobin proteins have proven to play an important role in Trypanolysis in human blood administered to rats. This explanation is in agreement with[16,17] who discuss the effect of human plasma containing different concentrations of apolipoprotein L-1 (APOL1) on the infected mice with *T. evansi*. It leads to an increase in longevity compared to the control group was 90%, 0% and 60% for the treated groups. They believed that this difference in the treatment efficiency is related to the level of APOL1 in plasmas. On the other hand,[14] concluded that human plasma was capable of eliminating or control parasitaemia to low levels, thus increasing the longevity of the animals, and added, the proteins found in human fluids might be responsible for the trypanolytic activity determining differences between rodents of the treated and non-treated groups.

Changes in hematological parameters due to treatment

The haematological characteristics of tested parameters in the different groups involved in the study are summarized in Table (2). Red blood-corpuscles (RBCs) and Hemoglobin (Hb) value decreased significantly in case of T group. The same conclusion as in case of Hb values was attained through changes of packed cell volume (PCV %). In contrast to the previous parameters, both mean cell volume (MCV) and mean corpuscular hemoglobin (MCH) increased significantly in untreated animals as compared with the negative control group. In the same contest,[18] indicated that the erythrocyte count (RBCs), PCV and Hb concentrations of *T. evansi* infected animals were decreased compared with those of the healthy rats. In infected rats, the increase in MCV values and the decrease in MCHC values indicated macrocytic hypochromic anaemia. However, this reduction in RBCs and PCV has been attributed to the release of hemolytic factors into the animal's blood by dead trypanosomes causing a destruction of erythrocytes and hence, reduction in PCV.[19] Also,[20] indicated that anaemia is a constant feature of trypanosome infections whose severity is linked to the level of parasitaemia.

The interplay of several factors acting either individually or synergistically contributes to the development of haemolytic anaemia in human and animal trypanosomiasis. Most common among these factors are erythrocyte injury caused by lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition.[21] No significant differences among the rats of T, Neg. and the other treated groups in RDW in the current study. The treatment with plasma proteins seemed to give highly significant increase in mean platelet volume MPV (in AB+ and A+ groups) and white blood cells WBCs (in A+ and O+ groups) as compared to DA and the negative control animals. Of these groups, animals in Neg., T, and the other treated groups didn't show statistically a significant difference in the tested values of platelets PLT. This coincides with the in vitro and in vivo results shown by[4,22] who showed that after the treatment with *Rosemarinus officinalis* extract, the tested haematological parameters of *T. evansi* infected animals were improved.

Biochemical parameters due to treatment

Changes in blood lipids

The mean levels of the tested blood lipids were summarized in Table (3). The present results indicated that the tested lipids cholesterol (Chol), triglyceride (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) generally tended to increase in response to the treatments with the two plasma proteins (AB, O) as compared with the negative control animals. In contrast, the tested Chol, TG, HDL and LDL and VLDL generally tended to reduce in the untreated animals and in response to the other tested treatments including the DA treated rats. The present findings are in conformity with[23] who demonstrated that the infection with *T. brucei* causes a significant decrease in the serum levels of Chol, HDL, TG, and LDL. On the other hand, the lowering of the serum lipids and Chol as observed in the present study could, partly, be the result of trypanosomal utilization of the molecules, and the continuous utilization of these molecules from the bloodstream could be a contributory factor to lowering of the serum levels of lipids and Chol.

Changes in Glucose and Calcium total

The mean values of serum Glucose and Calcium total in the infected, uninfected and treated animals are presented in Table (3). There were highly significant differences in the Calcium total values between the rates in Neg., untreated and other treated groups (F= 31.19). It is

known that blood-stream trypanosomes scavenge blood glucose for energy[24], which could cause hypoglycemia in the trypanosome-infected animal. Hypoglycemia can undoubtedly result in increased catabolism of lipids and Chol. in order to meet some strategic energy needs in the body of the host animal.[25] Consequently, this could lead to decrease in serum levels of these molecules as observed in the present study. Also, studies have demonstrated that low feed intake associated with trypanosomiasis[26] may affect blood levels of TG, HDL, and Chol. in infected animals.

Changes in blood proteins and kidney functions

Changes in rat plasma proteins affected by different tested treatments are presented in Table (4). The mean values of total protein and globulin were decreased significantly in case of rats treated with B+ as compared with the untreated rats. The obtained data showed that animals received DA drug as a treatment were suffering from the significant reduction in total protein and albumin as compared with the untreated animals. In support,[22] have been reported that in the infected control group there was a decrease in serum levels of ALP and albumin. This could be due to possible hepatic damage caused by the hemato-parasite induced in these animals. Unlike, the level of globulin was high. This could be described as possible antibody production. In the infected control group, hyperglobulinemia accompanied by hypoalbuminemia were observed and are similar to the observations made in a variety of mammal hosts infected by *T. evansi*.[27] In trypanosome infections, decreased serum albumin level has been reported.[28]

Changes in blood urea (BU), blood urea nitrogen (BUN) and creatinine (CR) in response to different tested treatments are presented in Table (4). In the present study, the kidney function in A, B, AB, O and DA groups (excepted DA group) did not show statistically significant differences when compared to the control group (Neg.). The decrease in CR levels when rats were given those treatments may be due to the ability of those treatments to provide some degree of protection to the kidneys during the course of the disease.[29]

Changes in liver enzymes

The evaluation of liver function was investigated in the experimental animals through the measurements of the liver enzymes including alanine transferase (ALT), aspartate transferase (AST); gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), Bilirubin total, Bilirubin direct and Bilirubin indirect as shown in Table (5). The activities of these enzymes are of clinical interest in the diagnosis of certain muscle and liver disorders.[30] In general, the present results showed that different enzymes levels in the animals are increased significantly by the infection with *T. evansi* parasite (T group) as compared with the uninfected animals (Neg. group). This agrees with other studies that reported elevated serum enzymes in trypanosomal infected animals.[4,31] The elevation of these enzymes is usually indicative of liver damage, being the major liver maker enzymes; or partly to cellular damage caused by lysis or destruction of the trypanosomes.[32] The results presented in Table (5) showed no significant differences of hepatic enzymes in A, B+, AB+, O+ and DA groups, excepted ALP which was statistically significantly decreased in DA, AB+, and O+ groups when compared to the control group (Neg.).

In general, the obtained data showed that the treatment with AB+ and O+ plasma proteins lead to the reduction in hepatic enzymes although that reduction was not statistically significant in most cases compared to negative animals. Also, similar results were obtained by[33] who found that although the serum levels of AST did not show a significant increase in treated animals compared to negative animals, they observed a decrease of AST in infected animals treated with the highest concentration of Curcumin. The reduction in the activity of liver enzymes, mainly ALT, indicates a better response of hepatocytes when facing the infection since the serum transaminase activities returns near to normal when the regeneration of the liver parenchyma occurs.[34] The present results revealed that there was a significant increase in the liver enzymes (except ALP), Bilirubin total, Bilirubin direct and Bilirubin indirect in the infected animals as compared with the healthy control rats. The high levels of total bilirubin in infected rats in the present study supports earlier observations in several trypanosome-infected animals.[35] It is suggestive of haemolytic anaemia which may be due to the activity of proliferating parasites and could also be associated with the inability of the liver to conjugate bilirubin.[36]

Histopathological changes after treatment

Brain

Histopathological examination of rat infected with *T. evansi* revealed demyelination of the brain, vacuolation, chromatolysis of neurons which appears as a ghost neuron and sever gliosis which clearly appear with neuronal phagia (Figure 1). The severity of lesion decreased during the treatment with different types of plasma protein, there was slight chromatolysis noted in rats of O+ and B+ groups, and appearance of neuronal oedema surrounding congested blood vessel in rats of AB+ group. These changes in the brain might be due to toxic substances released by the parasite and the pathological changes in the brain are due to constant irritation caused by the presence of parasites.[37] In addition, the other lesion including focal malacia of the brain, sever gliosis, neuronal phagia, chromatolysis of neurons and vacuolation of the brain may be the cause of nervous manifestation recorded in rat infected by *T. evansi* due to take a longitudinal section of the brain, dogs, and goats in *T. evansi* infection.[38,39,40]

Spleen

The histopathology of heart in different rat groups was illustrated in Figure 2. It is considered the most organ affected which characterized macroscopically by

splenomegaly. Microscopical examination revealed the clear appearance of splenic haemorrhage, depletion of white pulp and clear appearance of megakaryocyte (giant cells). Macroscopically, the lesion decreased only in case of treatment by O+ and beginning to the normal panoramic view of spleen with only slight depletion while other types of treatment from my point of view of no importance. As the clear appearance of haemosiderosis of spleen in case of AB+. The spleen is the most important organ that serves as the first line of defense mechanism.[41] Initial changes in spleen in rats of T group or the other tested groups may be due to immediate hypersensitivity to *T. evansi*, as described earlier.[42] Additionally, stimulation by the presence of *T. evansi* or their toxic metabolites results in varying degrees of anemic anoxia, which may induce splenic damage as observed by[43] in donkeys experimentally infected with *T. evansi*. During the progression of the disease, some histological changes such as hemorrhages, congestion and absence of germinal centers, haemosiderosis, increasing in follicular cells, focal necrosis and the formation of giant cells due to aggregation of histiocytes were also developed.[44]

Heart

The histopathology of heart in different rat groups was illustrated in Figure 3. Heart in the rat Neg. group showing cardiac muscles and coronary fat. It is clearly affected by trypanosome with the clear appearance of severing haemorrhage, cloudy swelling and necrosis of muscles with clear pyknotic nuclei as shown as in group AB with granulation of cytoplasm, and slightly in B+ group. Myocardiolysis was noticed in rats of A+ group. In addition, congestion was observed in rats of B+ group. Rat's heart of DA group showing severs hemorrhage and beginning of degenerative changes in cardiac muscles. In the present study, Myocardium revealed mild degenerative changes, interstitial oedema, degenerative changes in the heart may be due to anemia and hypoglycemia which also reported by[45] in experimentally infected rats with *T. evansi*. A mild to moderate interstitial myocarditis, in *T. congolense* infection in cattle[46], as well as which was observed in *T. brucei* infection in cattle and rabbits.[46] Also, mild to severe interstitial myocarditis, a large multifocal area of haemorrhage and mononuclear cells were observed in the epicardium of *T. evansi* infected buffalo.[38] Mild degenerative changes, interstitial oedema in myocardium were observed in mice infected with *T. evansi*.[37]

Liver

The histopathology of the liver in different rat groups was illustrated in Figure 4. Liver in the rat of Neg. group showing liver plates of hepatic cells and slight hemorrhage, also those of B+ group showing normal liver like healthy rats. Sever infiltration of inflammatory cells in DA and A+ groups. The presence of apoptotic cells was also observed in rats of A+ and AB+ groups. Liver congestion was observed in rats of AB+ and A+ groups. A newly formed bile duct was noted in rats of DA group. There was hepatic sinusoid dilatation in O+ group. However, Liver revealed necrosis, chronic inflammatory cells, with clear proteinaceous exudate in rats of T group. These results have partial agreement with that revealed congestion and haemorrhages of hepatocytes may be due to hypoglycemia leading to starvation of the cells and anoxia due to anaemia in *T. evansi* infected animals.[46] Similar to the obtained data, congestion in liver was observed in buffalo and in rats[38,48] infected with *T. evansi*. Also, congestion and necrosis of liver were observed in *T. brucei* and *T. congolense* infected sheep.[49]

Lung

The histopathology of the lung in different rat groups was illustrated in Figure 5. Alveolar emphysema was noted in the rat' lungs of Neg., DA, B+ and A+ groups with characteristic giant alveoli in DA and A+ groups only. Rats in DA, B+ and A+ groups suffering from interstitial pneumonia. Rats in T group suffering from suppurative bronchopneumonia. There was slight bronchitis, edema in rats of B+ group and catarrhal bronchitis desquamated epithelial cells inside the lumen were noticed in rats of O+ group. Rats in AB+ group suffering from peribronchial fibrosis and lymphocytic granuloma of the lung, respectively. Rats in DA group showed interstitial pneumonia, pre-bronchial lymphocytic aggregates and pneumoconiosis. In addition, all type of tested treatment failed to return lung to normal. Interstitial pneumonia found in the current study was also observed.[38] Histopathological changes in lungs were detected by[50] who observed marked cellular infiltration in lungs of *T. evansi* infected small African goats.

Kidney

The histopathology of the kidney in different rat groups was illustrated in Figure 6. Kidney of the rat in Neg. group showed normal appearance glomeruli and slight swelling of distal convoluted tubules. Rats in T group showed proteinaceous and edematous fluid in the cortex, while those in DA group showed hyalinization of glomeruli, atrophy of glomerular tuft. Microscopically, there was hydropic degeneration of distal convoluted tubules and necrosis (O+ group) which was clearly appeared in all groups as well as the negative group, slight congestion (A+ group). The obtained results showed that kidneys revealed tubular degeneration, congestion and cellular infiltration in most of the mice sacrificed and glomeruli were shrunken and degenerated. Also, kidneys in other cases characterized by hypercellularity and decrease in Bowman space, cloudy swelling of proximal and distal convoluted tubules and appear of hyaline and cellular cast inside tubules. It has been reported that changes in kidneys are mainly due to toxins produced by the parasite and accumulation of immune complexes which impair the structure and function of the kidney.[44,50,51]

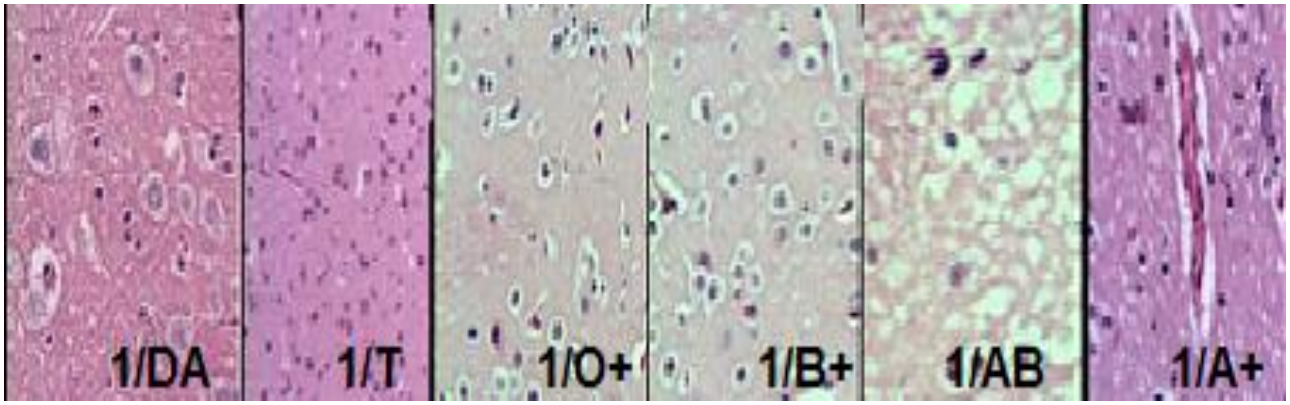

Figure 1: A photomicrograph of the brain tissues of Wistar rats after treatment against trypanosomiasis with diminazene aceturate (1/DA), and four plasma proteins (1/O+, 1/B+, 1/AB+ and 1/A+, respectively). 1/T represent infected but not treated rat group (H&E, X1000).

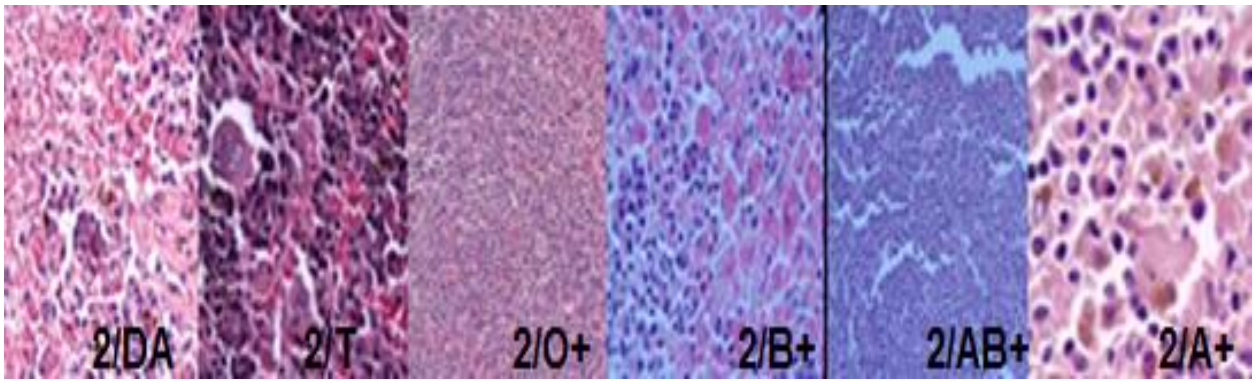

Figure 2: A photomicrograph of the spleen tissues of Wistar rats after treatment against trypanosomiasis with diminazene aceturate (2/DA), and four plasma proteins (2/O+, 2/B+, 2/AB+ and 2/A+, respectively). 2/T represent infected but not treated rat group (H&E, X1000).

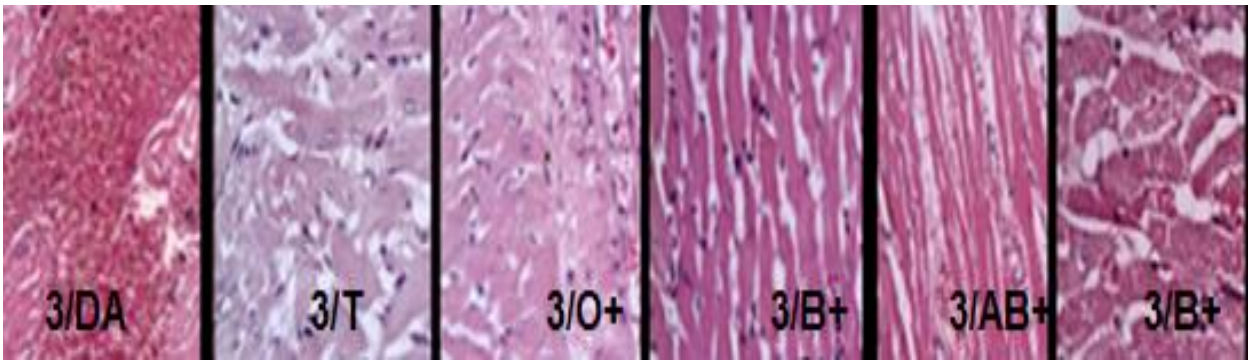

Figure 3: A photomicrograph of the heart tissue of Wistar rats after treatment against trypanosomiasis with diminazene aceturate (3/DA), and four plasma proteins (3/O+, 3/B+, 3/AB+ and 3/A+, respectively). 3/T represent infected but not treated rat group (H&E, X1000).

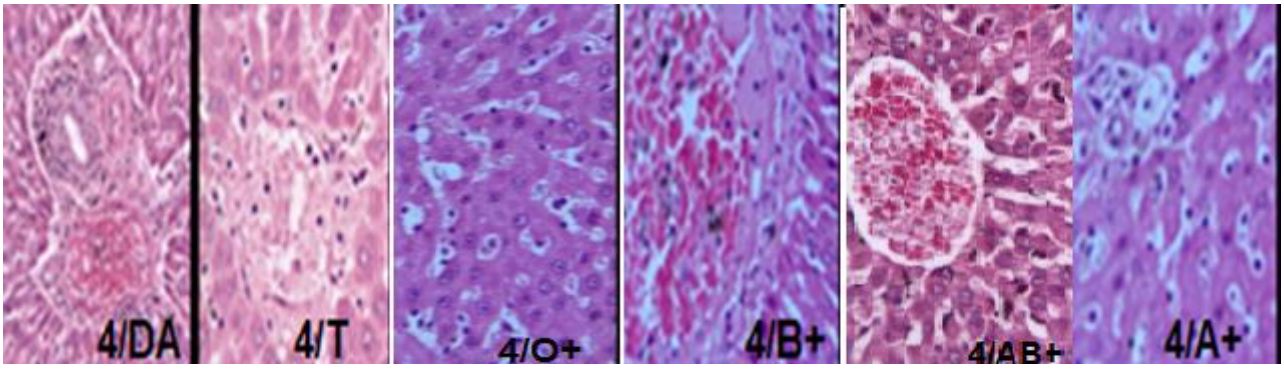

Figure 4: A photomicrograph of the liver tissues of Wistar rats after treatment against trypanosomiasis with diminazene aceturate (4/DA), and four plasma proteins (4/O+, 4/B+, 4/AB+ and 4/A+, respectively). 4/T represent infected but not treated rat group (H&E, X1000).

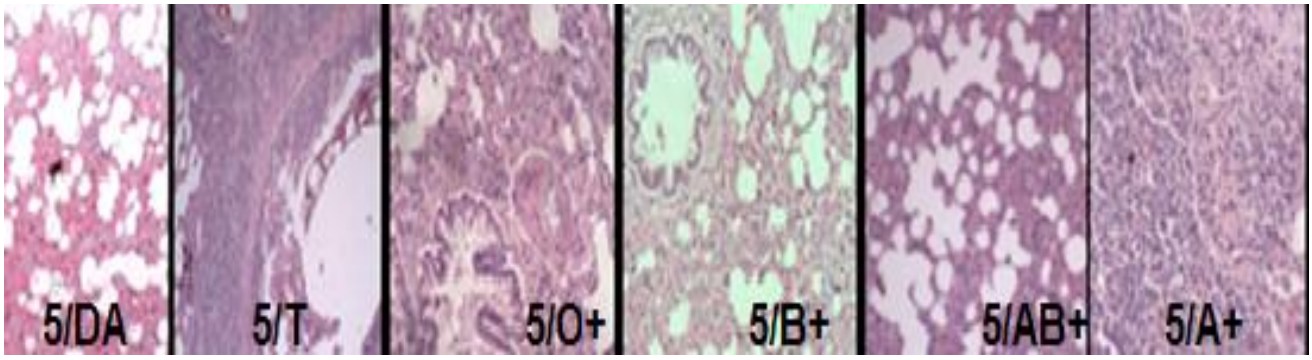

Figure 5: A photomicrograph of the lung tissues of Wistar rats after treatment against trypanosomiasis with diminazene aceturate (5/DA), and four plasma proteins (5/O+, 5/B+, 5/AB+ and 5/A+, respectively), whereas 5/T represent infected but not treated rat group (H&E, X,1000).

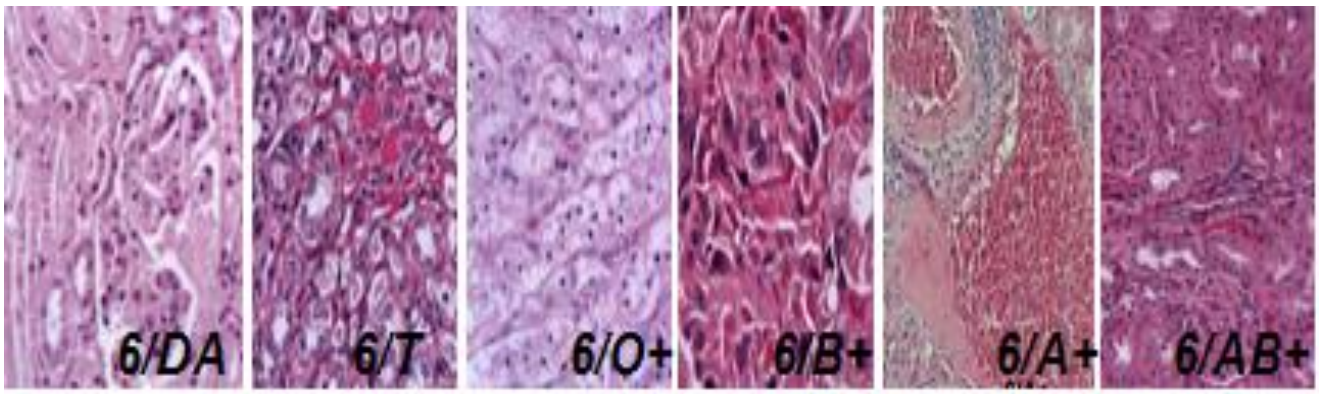

Figure 6: A photomicrograph of the kidney tissues of Wistar rats after treatment against trypanosomiasis with diminazene aceturate (6/DA), and four plasma proteins (6/O+, 6/B+, 6/A+, and 6/AB+, respectively), whereas 6/T represent infected but not treated rat group (H&E, X,1000).

Table 1: Means (±SE) of the longevity, mortality and therapeutic success rate (%) using treatment with plasmas of different blood groups and diminazene aceturate in rats experimentally infected with *T. evansi*.

Groups	Longevity (Mean ± SE).	Mortality	Success of treatment
Neg. (uninfected untreated)	42 ± 0^{d}	0%	-
T (infected untreated)	25 ± 4.946^{a}	83.33%	-
DA (treated with diminazene aceturate	40.83 ± 1.167^{d}	16.66%	83.34%
O+ (infected and treated with O+ plasma)	42.00 ± 0^{d}	0%	100%
B+(infected and treated with B+ plasma)	42.00 ± 0^{d}	0%	100%
A+(infected and treated with A+ plasma)	34.33 ± 1.406^{bc}	33.33%	66.67%
AB+(infected and treated with AB+ plasma)	31.67 ± 0.989^{b}	33.33%	66.67%

Table 2: Changes in hematological parameters due to treatment.

Groups	RBCs	HB	PCV	MCH	MCHC	WBCs	PLT
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Neg.	7.72±0.27^{cd}	15.37±0.53^{bc}	47.10±1.58^{bc}	19.96±0.59^{ab}	32.62±0.23^{cd}	8.52±1.05^{ab}	743.33±74.70^{a}
T	5.85±0.36^{ab}	11.31±0.73^{a}	34.92±2.20^{a}	25.21±1.76^{c}	32.36±0.05^{ab}	6.68±0.78^{a}	615.40±264.70^{a}
DA	6.59±0.67^{bc}	13.34±1.24^{ab}	41.02±3.73^{ab}	20.35±0.56^{ab}	32.50±0.07^{abcd}	6.41±0.89^{a}	565.10±99.55^{a}
A+	8.35±0.27^{d}	15.54±0.70^{bc}	47.61±2.11^{bc}	18.65±0.83^{a}	32.63±0.03^{cd}	18.85±1.85^{c}	755.25±70.09^{a}
AB+	6.63±0.57^{bc}	16.46±0.74^{c}	50.39±2.22^{c}	19.34±0.43^{ab}	32.67±0.03^{d}	8.84±1.32^{ab}	776.50±35.53^{a}
O+	7.02±0.57^{bcd}	13.80±0.88^{abc}	42.41±2.64^{abc}	20.00±1.41^{ab}	32.53±0.06^{bcd}	10.12±1.6^{ab}	612.33±44.77^{a}
B+	6.14±0.34^{abc}	13.7±0.92^{abc}	42.16±2.76^{abc}	22.32±0.86^{bc}	32.53±0.55^{bcd}	10.87±0.85^{ab}	498.00±23.51^{a}

Table 3: Changes in lipid contents and salts due to treatment.

Groups	Lipid contents					Glucose and Calcium	
	CHOL.	T.G	HDL	LDL	VLDL	Glucose	Calcium total
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Neg.	41.17±1.11^{c}	39.83±5.88^{b}	4.83±0.31^{c}	28.37±1.91^{efg}	7.97±1.18^{b}	122.67±5.86^{c}	9.77±0.09^{d}
T	21.80±2.71^{a}	15.50±2.0^{a}	2.40±0.51^{ab}	16.30±1.84^{a}	3.10±0.40^{a}	66.20±8.73^{a}	9.32±0.04^{b}
DA	30.60±3.37^{b}	21.60±2.48^{a}	3.20±0.37^{b}	23.08±2.75^{cde}	4.32±0.50^{a}	86.60±4.85^{b}	9.46±0.02^{bc}
A+	31.50±1.44^{b}	22.0±1.22^{a}	4.50±0.21^{c}	22.60±1.00^{cde}	4.40±0.245^{a}	86.25±5.56^{b}	9.55±0.03^{c}
AB+	43.25±1.49^{c}	81.50±1.19^{c}	4.75±0.25^{c}	22.20±1.07^{bcd}	16.30±0.24^{c}	94.50±1.94^{b}	10.08±0.10^{e}
O+	56.0±2.50^{d}	81.83±1.08^{c}	5.0±0.0^{c}	34.63±2.54^{h}	16.37±0.216^{c}	97.33±3.81^{b}	10.37±0.13^{f}
B+	31.33±2.14^{b}	42.83±6.09^{b}	3.30±0.44^{b}	19.47±1.105^{abc}	8.57±1.217^{c}	85.50±3.91^{b}	9.53±0.02^{c}

Table 4: Changes in total proteins and kidney functions due to treatment.

Groups	Total Proteins				kidney function			
	Total protein	Albumin	Globulin	A/G Ratio	BU	BUN	CR	UA
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Neg.	5.82±0.096^{ef}	3.72±0.048^{def}	2.10±0.053^{cd}	1.77±0.029^{cd}	20.67±0.80^{abc}	9.67±0.38^{abc}	0.36±0.01^{a}	2.75±0.21^{ab}
T	4.82±0.23^{bc}	2.66±0.12^{b}	2.16±0.12^{d}	1.24±0.01^{b}	47.20±10.29^{e}	22.07±4.81^{e}	1.18±0.29^{b}	4.06±0.14^{d}
DA	5.24±0.25^{bcd}	3.22±0.220^{c}	2.02±0.05^{bcd}	1.59±0.09^{c}	26.60±3.14^{bc}	12.44±1.47^{bc}	0.57±0.05^{a}	3.26±0.23^{c}
A+	6.03±0.24^{f}	3.98±0.18^{f}	2.05±0.07^{bcd}	1.94±0.04^{def}	15.0±1.25^{a}	7.13±0.59^{a}	0.38±0.042^{a}	2.55±0.05^{a}
AB+	6.03±0.10^{f}	4.10±0.09129^{f}	1.93±0.025^{bc}	2.13±0.05^{fg}	15.36±0.97^{a}	7.11±0.45^{a}	0.35±0.02^{a}	2.50±0.0^{a}
O+	6.08±0.10^{f}	4.14±0.12^{f}	1.94±0.042^{bc}	2.14±0.10^{fg}	17.40±0.91^{ab}	8.03±0.42^{ab}	0.38±0.02^{a}	2.50±0.0^{a}
B+	5.32±0.25^{cd}	3.47±0.17^{cde}	1.85±0.10^{bc}	1.88±0.06^{de}	24.75±1.50^{abc}	11.14±0.70^{abc}	0.50±0.04^{a}	2.79±0.13^{ab}

Table 5: Changes in Liver enzymes due to treatment.

Groups	ALT	AST	GGT	Bilirubin total	Bilirubin direct	Bilirubin indirect	ALP
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Neg.	30.33±1.98^{a}	92.00±10.15^{ab}	1.00±0.00^{ab}	0.20±0.00333^{ab}	0.07±0.00^{ab}	0.13±0.00^{abc}	36.83±0.40^{d}
T	112.40±35.91^{c}	474.00±133.69^{e}	1.64±0.35^{c}	0.28±0.03473^{cd}	0.10±0.01^{d}	0.18±0.02^{de}	31.60±0.51^{a}
DA	55.80±8.78^{ab}	174.60±25.89^{abcd}	1.12±0.08^{bc}	0.23±0.01860^{bc}	0.08±0.01^{bcd}	0.15±0.01^{bcd}	33.00±1.23^{ab}
A+	43.50±5.56^{ab}	122.50±26.26^{abc}	1.00±0.00^{ab}	0.19±0.00913^{ab}	0.06±0.01^{ab}	0.13±0.01^{abc}	37.00±0.41^{d}
AB+	30.50±1.04^{a}	89.50±1.26^{ab}	0.85±0.05^{a}	0.17±0.00645^{a}	0.05±0.01^{a}	0.11±0.00^{a}	34.50±0.87^{bc}
O+	31.17±2.90^{a}	91.67±5.34^{ab}	0.92±0.04^{ab}	0.18±0.00667^{ab}	0.06±0.00^{ab}	0.12±0.00^{abc}	34.33±0.58^{bc}
B+	45.83±5.70^{ab}	174.50±20.49^{abcd}	1.03±0.03^{bc}	0.23±0.01797^{bc}	0.07±0.00^{abc}	0.16±0.01^{cd}	36.00±0.37^{cd}

CONCLUSION

The in vitro test for antitrypanosomal activity revealed that all the tested plasma proteins at 0.001% cause disappearance of Trypanosoma. Treated groups with the plasma proteins (O+ and B+ groups) exhibited the highest antitrypanosomal effect against *T. evansi* followed by A+ and AB+ groups and the mean longevity of T group rats was of 25±4.95 days. For hematology parameters, RBCs, Hb and PCV% values decreased significantly, in contrast to both MCV and MCH that increased significantly and MPV (in AB+ and A+ groups) and WBC (in A+ and O+ groups) T as compared to DA and the negative control animals. For Biochemical analysis, Chol, TG, HDL, LDL and VLDL generally tended to increase in response to the treatments with the two plasma proteins (AB+, O+). Highly significant differences in the Calcium total values between untreated and treated groups and blood-stream trypanosomes scavenge blood glucose for energy causing hypoglycemia. The mean values of total protein and globulin were decreased significantly in treated group B+. DA drug caused a significant reduction in total protein and albumin as compared with the untreated animals. Results revealed changes in BU, BUN and CR

levels in response to different tested treatments**.** Enzymes levels in the animals are increased significantly by the infection, whereas ALP was significantly decreased in DA, AB+ and O+ groups when compared to the control group (Neg.). Histopathological examination evidenced that O+ treated animals followed by B+ did not suffer from abnormalities in contrast to A+ and AB+ treated animals.

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