Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 1622-1626

Received: 11-03-2019
Accepted: 15-04-2019

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Therapeutic efficacy of Isometamidium chloride in *Trypanosomiasis* affected cattle

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Abstract
To evaluate the efficacy of Isometamidium chloride, a total of ten (10) crossbred cattle suffering from surra were taken for this study. Trypanosomiasis was confirmed after microscopic detection of *Trypanosoma evansi* parasite in blood smears of the affected cattle. Affected cattle were treated with Isometamidium chloride injection (@ 0.5 mg/kg bwt IM) along with supportive therapy. Blood and serum samples collected from affected cattle on day 0 (pre-treatment), 3, 7 and 14 (post-treatment) were analysed for haematological and biochemical parameters respectively. Haematological alterations recorded in affected cattle were decreased Hb (8.36 g/dl ± 0.142), PCV (25.91% ± 0.467) and TEC (4.69 ×106/µL ± 0.132) indicating affected were afflicted with anaemia. The observed Leucopenia was accompanied by Lymphocytopenia, Eosinophilia, Neutrophilia and Thrombocytopenia. Similarly biochemical alterations observed in the affected animals were significantly decreased Glucose (34.81 mg/dl ±0.7), Total Protein (5.47 g/dl ± 0.078), Albumin (1.95 g/dl ± 0.055), Globulin (3.52 g/dl ± 0.043) and increased BUN (15.46 mg/dl ± 0.211), Creatinine (0.78 mg/dl ± 0.011 IU/L), ALT (33.9 ± 0.211 IU/L) and AST (123.2 ± 1.62; Table 2). A gradual disappearance of clinical signs with no parasitemia microscopically was recorded on 3rd day among all the affected cattle, along with a gradual restoration in the altered haemato-biochemical parameters on different post-treatment observation days. Hence, it was inferred that Isometamidium choride is very effective against *T evansi* infection in cattle.

Keywords: Isometamidium choride, *Trypanosoma evansi*, cattle, anaemia

1. Introduction
Surra is caused by a haemoprotozoan parasite named *Trypanosoma evansi* belonging to order of *Kinetoplastida* and family *Trypanosomatidae* that can infect both domestic animals and human [1]. The disease is prevalent over the tropics and sub-tropics of the globe with a wide host range [2-3]. Animal Trypanosomoses is a permanent constraint for livestock in Africa, Asia, and Latin America [2]. Transmission usually occurs through blood-sucking insects, especially *Tabanus* and *Stomoxys* [1] and incidence of disease occurs mostly from the month of July to November, following the onset of monsoons with breeding of transmitting flies [4].
Mostly the disease occurs in four forms *viz* per - acute form, acute, sub-acute and chronic. During acute form of infection, clinical signs like fever, anaemia, production losses, dullness with recumbency or staggering gait, laboured breathing, lacrimation, twitching of muscles often terminating in convulsions and death can be observed. Anaemia and hypoglycaemia are two most important pathological effects of *T evansi* infection [5]. Usually cattle and buffalo suffer from subclinical infection and revert to potent and clinical, on exposure to various stress conditions like hard work, transportation, inclement weather, malnutrition and other concurrent infections [6, 4]. Lack proper diagnosis and treatment failure causes economic losses in terms of both costs of treatment and production losses. Treatment mostly follows the use of antitrypanocide drug like diaminazene aceturate, quinapyramine sulphate and chloride and Isometamidium chloride for treatment and prophylactic use against trypanosomiasis in domestic animals along with supportive therapy [7]. But in India, diminazene aceturate, Quinapyramine sulphate and chloride (Antrycide Prosalt) are commonly being used for treatment and prophylactic use against *T. evansi* infection in domestic animals, where the drug resistance has rendered a severe and increasing problem in present days [8]. Due to availability of scanty literature on therapeutic efficacy of Isometamidium, the present study was conducted to assess the therapeutic efficacy of Isometamidium chloride in naturally affected trypanosomias in cattle through clinical examination, parasitological technique, and haematobiochemical parameters.

2. Materials and Methods

2.1 Study Area

The present study was conducted in and around Durg district of Chhattisgarh, an area which comes under the agro-climatic plane zone of the state between 20°54' and 21°32' North latitude & 81°10' and 81°36' East longitude. The climate of this district is of tropical type. Summer is a little bit hotter. Rainfall mostly occurs from the months of June to September. Hot, humid climate and early monsoon showers, which are the primary predisposing factors for fly reproduction and subsequent transmission of *Trypanosome* parasites. So the present study was conducted during the month of September 2018 at Anjora, Durg.

2.2 Sampling

A total of ten (10) cross bred cattle naturally infected with Trypanosomiasis and with history of inappetance or anorexia, fever, pallor mucous membrane were taken under study, which were confirmed through microscopic examination of stained blood smears. Blood samples were collected in EDTA and clot activator tubes for harvesting serum for analysis of haematological and biochemical parameters respectively.

2.3 Diagnosis of trypanosomiasis

Thick and thin blood slides were prepared immediately after each blood collection for detection of *Trypanosoma* parasite through microscopic technique. The blood smears were air-dried and fixed in methanol (99%), for 2–3 min, stained in field stain and examined at 100x magnification (oil immersion) for detection of *T. evansi* following standard protocols, as described by Murray *et al*. (1977) [9] and Paris *et al*. (1982) [10].

2.4 Estimation of haematological parameters

The blood samples collected from cattle (0 day; pre-treatment and 3 day, 7 day and 14 day; post-treatment) in EDTA vials were analysed through automated haematological analyser (Mindray company, model BC-2800Vet) following standard protocols for Hb (mg/dl), PCV (%), TEC ($\times 10^6$ /µl), TLC ($\times 10^3$ /µl). Differential Leucocytes Count (DLC%) was performed following standard procedure [11].

2.5 Estimation of biochemical parameters

Serum samples collected from cattle (0 day; pre-treatment and 3 day,7 day and 14 day; post-treatment) were processed for biochemical analysis like Serum Glucose (mg/dl), Total serum Protein (gm/dl), Serum Albumin (gm/dl), Serum Globulin (gm/dl), A/G ratio, AST (IU/L), ALT (IU/L), Serum Urea (mg/dl) and Creatinine (mg/dl) using semi-auto analyser (diaSIL- 100, Systonics India Ltd) by standard procedure as per the literature supplied with biochemical kits (Biolab diagnostic pvt ltd., Maharashtra).

2.6 Therapeutic efficacy

Animals were treated with Nyzom (Isometamidium chloride HCL injection; 0.5 mg/kg bwt IM) supplied by Intas Pharmaceutical Ltd along with supportive therapies. The drug efficacy was assessed through microscopic examination of stained blood slides on 3 days, 7 days and 14 days and haemato biochemical analysis on 0 (pre-treatment), day 3, 7 and 14 (post-treatment day) to establish the recovery by using the specific drug.

2.7 Statistical analysis

The results are presented as means ± SE for both the pre and post therapy values of infected and control groups. Analysis was carried out by using IBM SPSS software (version 20) for Duncan's Multiple Range Test (DMRT). $P < 0.05$ was considered as statistically significant.

3. Result

Trypanosomiasis in affected animals was confirmed by the morphology of *T. evansi*, as revealed slender and flagellated trypomastigote forms through examination of the field stained blood smears (Fig 1). Treatment was administered with isometamidium chloride @ 0.5 mg intramuscular along with supportive therapies. All treated animals were found parasitologically negative on 3rd day post treatment through stained blood smears and fever subsidised which was 105.52º±0.412 F might be due to administration of paracetamol injection. The haemato-biochemical parameters of *T. evansi* infected and healthy control cattle (0 day), post treatment (3rd, 7th and 14th day) are presented in Table 1 and 2. Macrocytic hypochromic anaemia was observed, through the parameter's variation, viz. reduction in HB, PCV, TEC, MCH and MCHC, but an increase in MCV. There was Leucocytopenia accompanied by Lymphocytopenia, Eosinophilia, Nutrophilia, Monocytosis, and Thrombocytopenia which indicates immunosuppression. Biochemical variations observed were decrease in blood Glucose, Total Protein, Albumin, Globulin and A/G ratio, while increase in BUN, ALT, AST and Creatinine, which were corrected on different day of post treatment period.

Post-treatment progress was observed through correction in the haematobiochemical alterations on 3rd, 7th and 14th day post-therapy. The anaemia was corrected significantly ($p<0.05$) through a substantial increase of Hb by the 14th day (post-treatment; 9.26 g/dl ± 0.146) from 0 day (pre-treatment; 8.36 g/dl ± 0.142), following an administration of Iron injection, vitamin B 12 and fluid therapies as supportive therapy. Also the altered TEC (4.69×106/µL ± 0.132) value recorded during the pre-treatment period significantly ($p\leq0.05$) increased by 7th day (4.89×106/µL ± 0.134) and 14th day (5.14×106/µL ± 0.132) of post-treatment period, but the altered PCV (25.91% ± 0.467) values was substantially increased by 3rd, 7th and normalised on 14th day (28.01% ± 0.437) post treatment. A significant Leucocytopenia (TLC; 8.78×103/µL ± 0.113) and Thrombocytopenia (Platelet counts; 306.5×103/µL ± 3.056) was corrected substantially on 3rd, 7th and normalised (TLC; 10.12×103/µL ± 0.115 and Platelet Counts; 402.8×103/µL ± 3.657) on 14th day post-treatment. The alterations in White Blood Cells (WBC) were found corrected on 14th day post treatment with a similar trend (Table 1). The biochemical alterations were also significantly ($p<0.05$) corrected by the 3rd, 7th and 14th day post treatment, where the altered blood Glucose (34.81 mg/dl ± 0.7) recorded pre-treatment period substantially increased on 3th, 7th and normalised on 14th day (49.54 mg/dl ± 0.38) of post treatment. Reduced Total Protein (5.47 g/dl ± 0.078) substantially corrected by 14th day (6.86 g/dl ± 0.101) post-treatment period accompanied by Albumin and A/G ratio in a similar trend, while the reduced Globulin (3.52 g/dl ± 0.043) and Creatinine (0.78 mg/dl ± 0.011) returned to normal value on 14th (3.64 g/dl ± 0.045) and 7th (0.64 mg/dl ± 0.012) day respectively. Similarly the altered BUN (15.46 mg/dl ±

0.211), AST (123.2 IU/L ± 1.62) and ALT (33.9 IU/L ±0.211) were substantially decreased on 3rd, 7th and normalised (BUN; 11.55 mg/dl ± 0.176, AST; 97.68 IU/L ± 0.52 and ALT; 32.96 IU/L ± 0.182) on 14th day (Table 2).

4. Discussion

During the study high fever was recorded in all the affected animals is a typical symptom of Trypanosomiasis in response to successive waves of parasitaemia, might be due to released endogenous pyrogens during infection so as to increase the body temperature set point in the hypothalamus [12]. Also the same has been recorded earlier by Kumar *et al*. (2012) [13]. All the affected animals were confirmed for *T. evansi* infection through microscopic examination of field stained peripheral blood smears. Since microscopic examination for detection of blood protozoa is the most accurate and reliable diagnostic tool in field condition. The typical structure viz. slender and flagellated Trypomastigote forms of *T. evansi* (fig 1) observed in all affected animals has also been previously detected by Mishra *et al.* (2017) [5] and Sivajothi *et al.* (2014) [14].

A significant anaemia recorded in all affected animals may pertain to extravascular haemolysis in the expanded active mononuclear phagocytic system of the host, followed by a drastic reduction of all red blood cell indices during successive waves of parasitaemia. Also, the mechanism of anaemia is complex and multifactorial in case of Trypanosomiasis [15]. During our study, all affected animals were recorded with lower Hb, PCV, TEC and TLC in is in accordance with the finding of Sivajothi *et al*. (2014) [14] and Hussain *et al*. (2016) [16]. A significant recorded leukopenia may be associated with the immunosuppressive action of Trypanosomes as well as exhaustion of immune system, usually due to wax and wear syndrome on the animal immune system caused by the ever changing variable surface glycoprotein of the infecting Trypanosomes [16]. However, the Trypanosomiasis affected cattle during our study showed a higher mean Neutrophil (%), higher mean Eosinophil (%) and lower mean Lymphocyte (%) and substantially increased till 14th day post-treatment observational period. This is in agreement with the findings of Mishra *et al.* (2017) [5] and Hussain *et al.* (2016) [16], which may indicate an initial enhanced immunological response followed by immunosuppressive effect of Trypanosome, influenced by its ever changing variable surface glycoprotein [18-19]. Often there occurs Eosinophilia condition in parasitic infections and is associated with immediate-type hypersensitivity reactions [14]. A mild Thrombocytopenia might be due to aggregation of platelets along with severity of parasitemia and indicative of immunological alterations [20]. All the affected animals were recorded with lower mean serum Albumin, and Total Protein are in agreement with previous findings of Dagnachew *et al.* (2014) [21] and Megahed *et al*. (2012) [22], might be due to increased hepatocellular damage accompanying hypoxia [23]. Also higher mean serum AST and ALT concentration (Units/L) recorded in all affected cattle are in accordance with the findings of Yusuf *et al*, (2012) [24] and Hussain *et al.* (2016) [16], which might be due to centrilobular degeneration caused by hypoxia and severe oxidative stress induced by the parasite [16]. The catchy finding *viz*. hypoglycaemia recorded among affected cattle during pre-treatment period is in agreement with the findings of Sazmand *et al.* (2011) [25], since Trypanosomes are voracious consumers of host glucose utilizing them for their metabolism, leading to hypoglycemic condition [5].

The drug Isometamidium chloride was found to effectively reduce parasitaemia by the 3rd day of post treatment from peripheral blood circulation along with significant ($p<0.05$) restoration of altered haemato-biochemical parameters to their normal levels on 14th day of post treatment (Table 1 and 2), which also might be supplemented with the supportive therapies administered to the animals. A similar finding has previously been recorded by Singh *et al*. (2012) [26]. Isometamedium compounds possess both curative and prophylactic properties and the only drug available, after the discontinuation of quinapyramine compounds for induction of multidrug resistances and toxicity, but present day treatment constrain is due to evolution of multiple-drug resistant trypanosomes against other trypanoside drugs also [27]. A huge economic loss occurs due to Surra in livestock, since treatment costs and failures are high; production losses etc significantly affect both the farmers and livestock, which create a need to establish an effective drug and reduction of cost intervention with respect to therapies. Also further research is required to establish the status of disease in the whole state and its control. Due to its variable surface glycoprotein, vaccine could not be successfully developed [2]. So control and prevention can be achieved through vector control and chemoprophylaxis. The major constraint in production and development of an antitypanosomal drug is its high cost.

Table 1: Haematological findings of T. *evansi* affected cattle on different days of pre and post treatment (mean ± SE)

S. No	Parameters	Pre-treatment	Post-treatment		
		Day 0	Day 3	Day 7	Day 14
1	Hb (g/dl)	8.36 ^{a}±0.142	8.56 ^{b}±0.142	8.76 ^{c}±0.142	9.26 ^{d}±0.146
2	PCV (%)	25.91 ^{a}±0.467	26.39 ^{b}±0.452	26.84 ^{c}±0.449	28.01 ^{d}±0.437
3	MCV (fl)	55.44 ^{a}±0.723	55.27 ^{ab}±0.706	55.07 ^{b}±0.711	54.66 ^{c}±0.711
4	MCH (pg)	17.89 ^{a}±0.259	17.93 ^{ab}±0.255	17.98 ^{ab}±0.255	18.07 ^{b}±0.267
5	MCHC (g/dl)	32.27 ^{a}±0.13	32.44 ^{b}±0.117	32.64 ^{c}±0.098	33.06 ^{d}±0.122
6	TEC ($\times 10^{6}$/µL)	4.69 ^{a}±0.132	4.79 ^{ab}±0.133	4.89 ^{b}±0.134	5.14 ^{c}±0.132
7	TLC ($\times 10^{3}$/µL)	8.78 ^{a}±0.113	9.14 ^{b}±0.115	9.51 ^{c}±0.12	10.12 ^{d}±0.115
8	Lymphocyte (%)	40.1 ^{a}±0.567	56.2 ^{b}±01.218	60 ^{c}±0.83	63.2 ^{d}±0.39
9	Eosinophil (%)	8.3 ^{a}±0.335	3.9 ^{b}±0.277	3 ^{c}±0.211	2.5 ^{d}±0.167
10	Neutrophils (%)	48.4 ^{a}±0.452	37.2 ^{b}±1.153	34.6 ^{c}±0.846	32.5 ^{d}±0.428
11	Basophil (%)	0	0	0	0
12	Monocyte (%)	3.2 ^{a}±0.294	2.7 ^{b}±0.213	2.4 ^{b}±0.267	1.8 ^{c}±0.249
13	Platelet ($\times 10^{3}$/µL)	306.5 ^{a}±3.056	325.4 ^{b}±3.11	351.3 ^{c}±3.596	402.8 ^{d}±3.657

N.B.: a, b, c and d superscript indicate significant difference between different day (0, 3rd, 7th and 14th). Same superscript indicates for no significant difference between the different days.

Table 2: Biochemical findings of *T. evansi* affected cattle on different days of pre and post treatment (mean ± SE)

S. No.	Parameters	Pre-treatment	Post-treatment		
		0 Day	3 Day	7 Day	14 Day
1	Glucose (mg/dl)	34.81^{a}±0.7	43.61^{b}±0.68	48.84^{c}±0.43	49.54^{d}±0.38
2	BUN (mg/dl)	15.46^{a}±0.211	14.27^{b}±0.253	12.31^{c}±0.22	11.55^{d}±0.176
3	AST (IU/L)	123.2^{a}±1.62	106.72^{b}±1.92	98.99^{c}±0.75	97.68^{d}±0.52
4	ALT (IU/L)	33.9^{a}±0.211	33.6^{b}±0.186	33.28^{c}±0.165	32.96^{d}±0.182
5	Creatinine (mg/dl)	0.78^{a}±0.011	0.72^{b}±0.012	0.64^{c}±0.012	0.61^{c}±0.008
6	Total Protein (g/dl)	5.47^{a}±0.078	6.06^{b}±0.078	6.64^{c}±0.108	6.86^{d}±0.101
7	Albumin (g/dl)	1.95^{a}±0.055	2.5^{b}±0.059	3.05^{c}±0.099	3.22^{d}±0.093
8	Globulin (g/dl)	3.52^{a}±0.043	3.56^{ab}±0.041	3.59^{bc}±0.045	3.64^{c}±0.045
9	A/G ratio	0.55^{a}±0.015	0.7^{b}±0.017	0.85^{c}±0.03	0.89^{c}±0.03

N.B.: a, b, c and d superscript indicate significant difference between different day (0, 3rd, 7th and 14th). Same superscript indicates for no significant difference between the different days.

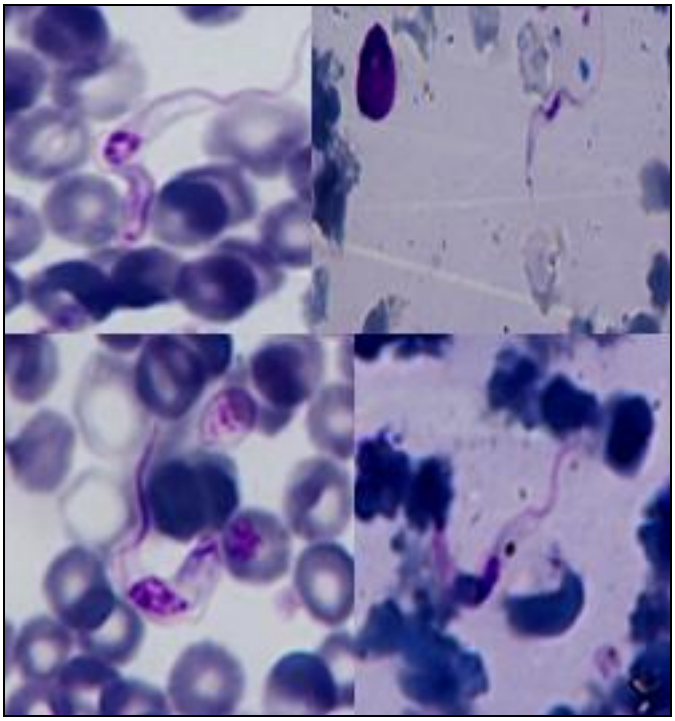

Fig 1: Microscopic view of *T. evansi* under 100x (oil immersion)

5. Conclusion

High fever accompanied by significant hypoglycaemia and hypochromic macrocytic anaemia recorded is the catchy clinical findings of Trypanosomiasis. The drug Isometamidium chloride was very effective to clear the parasite from blood by the 3rd day of treatment in treated animals. Specific treatment and supportive therapy resulted amelioration of clinical signs by 3rd day post treatment and restoration of altered haemato-biochemical parameters to their normal level by 14th day of treatment.

6. Acknowledgement

The authors are highly thankful to the Deputy Directors, VS, AVFO and attendants for their help during sample collection. Thanks are also due to Dean, College of veterinary Science & AH, Anjora for providing facilities to carry out this work. This is a part of Ph.D thesis work of the first author.

7. Reference

1. Dyary HO, Arif AH, Sharma RSK, Rasedee A. Antitrypanosomal and cytotoxic activities of selected medicinal plants and effect of *Cordylineterminalis* on trypanosomal nuclear and kinetoplast replication. Pak. Vet. J. 2014; 34:444-448.
2. Desquesnes M, Dargantes A, Lai DH, Lun ZR, Holzmuller P, Jittapalapong S. *Trypanosoma evansi* and Surra: A Review and Perspectives on Transmission, Epidemiology and Control, Impact, and Zoonotic Aspects. BioMed Research International. 2013; 2013:1-20.
3. Da Silva AS, Wolkmer P, Costa MM, Tonin AlA, Eilers TL, Gressle LT, *et al*. Biochemical changes in cats infected with *Trypanosoma evansi.* Veterinary Parasitology. 2010; 17:48-52.
4. Rani NL, Suresh K, Rajesh K. A retrospective study on clinico-epidemiological aspects of trypanosomiasis in buffaloes. Inter. J Vet. Sci. 2015; 4(2):97-100.
5. Mishra RR, Senapati SK, Sahoo SC, Das MR, Sahoo G, Patra RC. Trypanosomiasis induced oxidative stress and hemato-biochemical alteration in cattle. Journal of Entomology and Zoology Studies. 2017; 5(6):721-727.
6. Chanie M, Adula D, Bogale B. Socio-Economic Assessment of the Impacts of Trypanosomiasis on Cattle in Girja District, Southern Oromia Region, Southern Ethiopia. Acta Parasitologica Globalis. 2013; 4(3):80-85.
7. Juyal PD. Newer Perspectives in the Diagnosis and Control of Trypanosomosis (Surra) in Domestic Livestock in India. TROPMED - Internationale Wissenschaftliche Publikationen, 2011, 1-13.
8. Ponnudurai G, Sivaraman S, Rani N, Veerapandian C. An outbreak of trypanosomosis in buffaloes caused by diminazene resistant *Trypanosoma evansi*. Buffalo Bulletin. 2015; 34(1):1-4.
9. Murray MM, Murray PK, McIntyre WIM. An improved parasitological technique for the diagnosis of African Trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1977; 71:325-328.
10. Paris J, Murray M, McOdimba FA. Comparative evaluation of the parasitological methods currently available for the diagnosis of Trypanosomiasis in cattle. Acta. Tropica. 1982; 37:307-316.
11. Jain NC. Schalm's Veterinary Hematology. 4th ed. Lea and Febiger, 600. Washington square, Philadelphia, USA, 1986.
12. Pathak AK. Effect of *Trypanosoma spp.* on Nutritional status and performance of livestock. Veterinary World, 2009; 2(11):435-438.
13. Kumar H, Gupta MP, Sidhu PK, Mahajan V, Bal MS, Kaur K, *et al*. An outbreak of acute Trypanosoma evansi infection in crossbred cattle in Punjab, India, Journal of Applied Animal Research. 2012; 40(3):256-259.
14. Sivajothi S, Reddy BS, Kumari KN, Rayulu VC. Haematological changes in Trypanosoma evansi infected

cattle. International Journal of Scientific World. 2014; 2(1):27-30.
15. Mbaya A, Kumshe H, Nwosu CO. The Mechanisms of Anaemia in Trypanosomosis: A Review, Anemia, Dr. Donald Silverberg (Ed.), ISBN: 978-953-51-0138-3, In Tech. 2012, 269-282. Available from: http://www.intechopen.com/books/anemia/the-mechanisms-of-anaemia-in-trypanosomosis-a-review
16. Hussain R, Khan A, Abbas RZ, Ghaffar A, Abbas G, Ali F. Clinico-Hematological and Biochemical Studies on Naturally Infected Camels with Trypanosomiasis. Pakistan Journal of Zoology. 2016; 48(2):311-316.
17. Desquesnes M, Holzmuller P, Lai DH, Dargantes A, Lun ZR, Jittaplapong S. *Trypanosoma evansi* and Surra: A Review and Perspectives on Origin, History, Distribution, Taxonomy, Morphology, Hosts, and Pathogenic Effects. BioMed Research International. 2013; 2013:1-22.
18. Adeyemi OS, Sulaiman FA. Biochemical and morphological changes in Trypanosoma brucei brucei-infected rats treated with homidium chloride and diminazene aceturate. Journal of basic and clinical physiology and pharmacology. 2012; 23(4):179-183.
19. Pays E, Vanhollebeke B, Vanhamme L, Paturiaux-Hanocq F, Nolan DP, Pérez-Morga D. The trypanolytic factor of human serum. Nature Reviews Microbiology. 2006; 4(6):477-486.
20. Singh V, Singla LD. Trypanosomosis (Surra) in Livestock. Inbook: Veterinary Parasitology in Indian Perspective, Edition: 1st, Chapter: 11. Publisher: Satish Serial Publishing House, Delhi. Editors: Rajesh Katoch, Rajesh Godara, Anish Yadav (Ed. 2013, 305-330.
21. Dagnachew S, Terefe G, Abebe G, Barry DJ, Goddeeris BM. Comparative biochemical changes in young Zebu cattle experimentally infected with *Trypanosoma vivax* from tsetse infested and non-tsetse infested areas of northwest Ethiopia. Veterinary parasitology. 2014; 205(3):451-459.
22. Megahed GA, Ellah MRA, Abdel-Rady A. Comparative biochemical studies on natural *Trypanosoma evansi* infection in she-camels. Comparative Clinical Pathology. 2012; 21(5):1121-1124.
23. Abo-Aziza FAM, Ashry HM, Nassar SA. Haematological and Biochemical Alterations in Sub Clinically Affected Dromedary Camels with *Trypanosoma evansi* During Breeding Season in Egypt. Journal of Chemical and Pharmaceutical Sciences. 2017; 10(3):1326-34.
24. Yusuf AB, Umar IA, Nok AJ. Effects of methanol extract of Vernonia amygdalina leaf on survival and some biochemical parameters in acute Trypanosoma brucei brucei infection. African Journal of Biochemisty. Research. 2012; 6(12):150-158.
25. Sazmand A, Rasooli A, Nouri M, Hamidinejat H, Hekmatimoghaddam S. Serobiochemical alterations in subclinically affected dromedary camels with Trypanosoma evansi in Iran. Pakistan Veterinary Journal. 2011; 31(3):223-226.
26. Singh R, Gupta SK, Upadhyay S. Chemotherapy and Evaluation of Drug Efficacy in Equines Infected with *T. evansi* with Antrycide Prosalt and Isometamedium Chloride. Veterinary Practitioner. 2012; 13(2):139-42.
27. Giordani F, Morrison LJ, Rowan TG, DE Koning HP, Barrett MP. The animal trypanosomiases and their chemotherapy: a review. Parasitology. 2016; 143:1862-1889.