

## THERAPEUTIC EFFICACY EVALUATION OF COMMONLY USED ANTITRYPANOSOMAL DRUGS IN NATURALLY INFECTED BUFFALOES

Ashish Pratap Singh, Arvind Kumar Tripathi\*, Rudra Pratap Pandey and Ashish Srivastava

### ABSTRACT

In present investigation therapeutic efficacy of three most commonly used anti-trypanosomal drugs (isometamidium chloride hydrochloride, diminazine aceturate, and quinapyramine sulphate) were studied. Evaluations of therapeutic efficacy were assessed done on the basis of percent recovery assessment, hematological and biochemical alteration on day 0, day 7<sup>th</sup> and day 14<sup>th</sup> post treatment. All three drugs were found effective against the trypanosomosis in buffalo but the extent of improvement in terms of hematological values, biochemical values and percent recovery was observed maximum in the treatment with isometamidium chloride hydrochloride followed by diminazine aceturate and least with the quinapyramine sulphate.

**Keywords:** *Bubalus bubalis*, buffaloes, therapeutic efficacy, anti-trypanosomal drugs, percent recovery, trypanosomosis

### INTRODUCTION

Trypanosomosis is economically one of the most important hemoprotozoan diseases of

bovines in India. This is caused by unicellular, flagellated protozoa of the genus *Trypanosoma*. It is an important vector born disease occurring in tropical and subtropical countries including India (Da-silva *et al.*, 2009). Currently available drugs for treatment and control of Trypanosomosis in India are diminazine aceturate, quinapyramine prosalt, and Isometamidium chloride hydrochloride. Till now many studies were conducted regarding comparative efficacy of antitrypanosomal drugs and variable results were reported by different workers. Therefore, keeping in view of the above facts present study were planned to investigate above mentioned antitrypanosomal drugs in naturally infected buffaloes with aim to evaluate their therapeutic efficacy.

### MATERIALS AND METHODS

The study was performed at teaching veterinary clinical complex, DUVASU, Mathura from June 2013 to May 2014. The comparative efficacies of three anti-trypanosomal drugs were studied by using these drugs in standard dosage regimen in the treatment of positive cases. These animals were grouped in following four group having six buffaloes in each groups (n=6).

---

Department of Veterinary Medicine College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Uttar Pradesh, India, \*E-mail: arvindvet04@rediffmail.com

Group A: Positive cases of trypanosomosis treated by isometamidium chloride hydrochloride 0.8 mg/kg body wt. along with supportive therapy.

Group B: Positive cases of trypanosomosis treated by diminazene aceturate 7 mg/kg body wt. along with supportive therapy.

Group C: Positive cases of trypanosomosis treated by quinapyramine sulphate 5 mg/kg body wt. along with supportive therapy.

Group D: Apparently healthy buffaloes were kept as control.

Supportive therapy was done as per need of the animal of each group like fluid therapy, antipyretics, haematinics, antibiotics, vitamins and minerals etc.

The therapeutic efficacy of above anti-trypanosomal drugs were evaluated on the basis clinical improvement in terms of disappearance of clinical signs, parasitological examination and alterations in the hemato-biochemical parameters on day 0, day 7<sup>th</sup> and day 14<sup>th</sup> post treatment. All the positive cases of each group were thoroughly examined by clinical examination and parasitologically by blood smear examination on the day 0 (pretreatment), 7<sup>th</sup> and day 14<sup>th</sup> after treatment. Percent recovery assessment was done on the basis of improvement in terms of disappearance of clinical signs and number of blood smear negative buffaloes on the day 7<sup>th</sup> and day 14<sup>th</sup> after treatment. Those animals which were found blood smear positive with clinical signs on the day 7<sup>th</sup> after treatment were again treated with the same anti-trypanosomal drug along with supportive therapy.

$$\% \text{ Recovery} = n/6 \times 100$$

n = no of blood smear negative buffaloes within a group after treatment.

Hematological and biochemical analysis of all the positive cases of each group were done on the day 0 (before), 7<sup>th</sup> and 14<sup>th</sup> day after treatment. The blood was aseptically collected from the jugular vein, using a 20 gauge needle for each animal separately in 5 ml blood collection vials containing sodium ethylene diamine tetra acetate (EDTA) as an anticoagulant 1 mg /ml. Following parameters viz hemoglobin, TEC, PCV, TLC, and DLC were assessed using hematology auto analyzer (Diatron's Abacus Hematology Analyzer, Wien, Australia).

The blood for serum required for biochemical estimations were collected in a 10 ml capacity test tubes with no anticoagulant and were allowed to stand undisturbed in slant position for about 3 to 4 h. The clots were retracted and the serum separated after rapid centrifugation. Extreme care was taken to prevent hemolysis. The serums collected were stored in a deep freeze at -20°C in glass vials, which were properly capped and labeled till analysis. Various biochemical parameters like serum total proteins (g/dl), albumin(g/dl), creatinine(mg/dl), BUN (mg/dl) and the enzymatic activities like alanine amino transferase (units/L), aspartate amino transferase (units/l), alkaline phosphatase (units/l) were done with the help of BS-120 Chemistry Analyzer (2007 to 2010 Shenzhen Mindray Biochemical Electronics Co. Ltd.) using Span diagnostic kits (Span Diagnostics Ltd, Sachin, Surat, India). Blood glucose (mg/dl) was estimated with the help Glucometer using blood glucose test strips (Gluco Chek, Aspen Diagnostics (P)) LTD. Delhi-33, India). Statistical analysis of all the data to test significance of means was done as per the method described by IBM, SPSS Statistics 20.

## RESULTS AND DISCUSSION

### Therapeutic efficacy on the basis of percent recovery assessment

Percent recovery assessment was done on the basis clinical improvement in terms of disappearance of clinical signs and parasitological examination in present study, the percent recovery shown by the isometamidium chloride hydrochloride (Group A) on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment was found to be 100%. The percent recovery shown by diminazine acetate (Group B) on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment was found to be 83.33% and 100% respectively. The percent recovery shown by quinapyramine sulphate (Group C) on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment was found to be 50% and <100% respectively (Table 1).

The present findings regarding the effect of isometamidium hydrochloride on *T. evansi* is found almost similar with the previous findings by Kumar *et al.* (2009) who studied the effect of isometamidium hydrochloride on *T. evansi* infections in rats. The findings of the present studied are in agreement with the findings of Awa *et al.* (2006) who reported that isometamidium can be used both for therapeutic and prophylaxis of bovine trypanosomiasis. Toro *et al.* (1983) also found that the drug was found to be highly effective against *T. vivax* followed by *T. evansi* infection in sheep and cattle. The findings reported in present investigation does not corroborates with the findings reported by Ajayi *et al.* (2013) Who reported that diminazine acetate was more effective than isometamidium chloride. Howes *et al.* (2011) found that a single dose of drug were not effective to control the *T. evansi* infection, it can be correlated with the possible causes of present findings where one animal of Group B and three animals of Group C were found again blood smear

positive after the day 7<sup>th</sup> of treatment with their respective drugs. This could also be due to the facts that indiscriminate use of diaminazine and quinopyramine could leads to development of resistance among trypanosomes (Pathak and Singh, 2005). Pholpark *et al.* (1984) also reported that blood smear examination could be found positive for trypanosomes after treatment with diminazene at 5 mg/kg. However, Gill (1991) considered diminazene as sanative drug and found it to be very effective in treatment of trypanosomiasis in buffaloes.

### Therapeutic efficacy on the basis of hematological alterations

In present study it was found that there was a significant reduction ( $P < 0.05$ ) in hemoglobin concentration, packed cell volume and total erythrocyte count (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control (Group D). There was a significant increase ( $P < 0.05$ ) in the hemoglobin concentration, packed cell volume and total erythrocyte count at the day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. Therefore in terms of improvement in treated groups of buffaloes best recovery was assessed in treatment with isometamedium followed by diaminazine and quinopyramine (Table 2).

The present findings of decrease in Hb, PCV and TEC in cases of trypanosomiasis are in agreement with the findings of Hilali *et al.* (2006); Takeet *et al.* (2009); Abeer *et al.* (2011); Fetehanegest *et al.* (2012) that inhibition on the haemopoietic system by the toxins liberated by the trypanosomes resulting in failure in production of the RBCs. It had been also reported that the attachment of

trypanosome antigen to RBC, may increase the cell susceptibility to erythrophagocytosis, which may be further increased by the union of surface absorbed antigen with antibody could decrease RBC, PCV and Hb values (Herbert and Inglis, 1973). This. Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of reduced glutathione on the surface of the red blood cell (Akanji *et al.*, 2009; Kumar *et al.*, 2011) reported significant reduction in the Hb, PCV, and TEC in trypanosomosis and also revealed that after treatment there was significant increase in all these values.

The total leukocyte count was significantly higher ( $P<0.05$ ) (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control. There was a significant decrease ( $P<0.05$ ) in the total leukocyte count at the day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. Therefore in terms of improvement in treated groups of buffaloes best recovery was assessed in Group A followed by Group B and minimum in Group C. There was a significant increase ( $P<0.05$ ) in neutrophils (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control. There was a significant decrease ( $P<0.05$ ) in the neutrophils at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. Therefore in term of improvement in neutrophils percentage in treated groups of buffaloes, best recovery was assessed in treatment with isometamedium followed by diaminazine and quinopyramine.

There was a significant decrease ( $P<0.05$ )

in lymphocytes (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control. There was a significant increase ( $P<0.05$ ) in the lymphocytes at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. Therefore, in term of improvement in lymphocytes percentage in treated groups of buffaloes, best recovery was assessed in treatment with isometamedium followed by diaminazine and quinopyramine.

The increased WBC and neutrophil counts are indicative of increased host defence against the infection; contribute to the development of phagocytes and antibodies against the recognizable antigens of parasite origin (Kumar *et al.*, 2012). The observed decrease in the lymphocyte count could be a result of the corresponding increase of neutrophil count during the infection. Leucocytosis was reported to be associated with *T. evansi* infection in buffalo calves by Walia *et al.* (1996); Kulkarni *et al.* (1997). Leukocytosis and lymphocytopenia found in our study are in agreement with the findings of Kulkarni *et al.* (1998); Abeer *et al.* (2011).

There was no any significant difference ( $P>0.05$ ) in basophils, monocyte and eosinophils percentage (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control. There was no significant change ( $P>0.05$ ) in the basophils, monocyte and eosinophils percentage at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups. Therefore, in present study no any variation in term of percent basophils, monocyte and eosinophils was seen in all the treated groups. The findings of present investigation are contrary to the findings earlier reported by Ugochukwu (1986) who recorded slight reduction in the number of eosinophils, monocytes

and basophils in trypanosomiasis in cattle. From present study it can be concluded that all three drugs were effective against the trypanosomiasis but the extent of improvement in the hematological values were highest with isometamidium chloride hydrochloride followed by diminazene aceturate and least with the quinapyramine sulphate.

### **Therapeutic efficacy on the basis of biochemical alterations**

Biochemical evaluation gives an indication of the functional state of the various body organs and biochemical changes in body fluids that result from infections depend on the species of the parasite and its virulence (Anosa, 1988a). There was no significant alteration in serum total protein (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control (Table 3). There was no significant change ( $P>0.05$ ) in the serum total protein at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups. Therefore, in present study no any variation in term of serum total protein values were seen in all the treated groups. Findings of present investigations are in agreement with the observations earlier made by Monzon *et al.* (1990); Anene *et al.* (2011).

There was a significant reduction ( $P<0.05$ ) in the serum albumin concentration (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control. However, there was a significant increase ( $P<0.05$ ) in the albumin concentration at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. There was a significant increase ( $P<0.05$ ) in globulin concentration (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control. However, there was a significant decrease ( $P<0.05$ )

in the globulin concentration at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. These findings of present investigations are in accordance with findings of Awobode (2006); Abenga and Anosa (2005) reported decrease in albumin and raised globulin level in their study. Hilali *et al.* (2006) found significant elevation in the globulin level which is similar with the present finding. The elevated level in the globulin could be due to elevation in the gamma globulin, which was secreted as immunological response against *T. evansi* (Keniko, 1997). Taiwo *et al.* (2003) studied the comparative plasma biochemical changes in trypanosome positive sheep and found elevation of total plasma protein and globulin levels with resultant decrease in albumin: globulin ratio. Values of serum albumin and serum globulin were found to be significantly elevated after treatment with different drugs on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment in all the groups but best recovery observed in treatment with isometamedium followed by diaminazine and quinopyramine.

In present investigation there was a significant increase ( $P<0.05$ ) in serum BUN concentration (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control were recorded. However, there was a significant decrease ( $P<0.05$ ) in the BUN concentration at a day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. The increase in values of BUN could be due to body catabolic breakdown of proteins as a result of the fever (Kadima *et al.*, 2000; Coles, 1986). Elevation in the urea level in the present study is in consonance with previous findings by Abeer *et al.* (2011); Takeet *et al.* (2009).

Values of BUN were found to be significantly decreased after treatment with different drugs on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment in all the groups but best recovery observed in treatment with isometamedium followed by diaminazine and quinopyramine.

Significant increase ( $P<0.05$ ) in serum creatinine concentration (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control were recorded. However, there was a significant decrease ( $P<0.05$ ) in the serum creatinine concentration at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in Group A and Group B and no significant decrease in creatinine concentration was recorded with Group C. Therefore, in term of improvement in serum creatinine concentration in treated groups of buffaloes, best recovery was assessed in Group A followed by Group B and no improvement in Group C. In present study the elevated level of creatinine, in the positive cases before treatment are found in agreement with the findings of Abeer *et al.* (2011); Omejea *et al.* (2012). The elevation in the serum creatinine level would seem to suggest renal injury and associated glomerular dysfunction (Anosa, 1988a; Anosa, 1988b). Values of serum creatinine were found to be significantly decreased after treatment with isometamedium and diaminazine on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment but best recovery observed in treatment with isometamedium followed by quinopyramine. However, no significant decrease was noticed in quinopyramine treated group.

In present investigation there was a significant increase ( $P<0.05$ ) in serum ALT, AST and ALP concentration (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control were recorded. However, there was a significant decrease ( $P<0.05$ ) in the

serum ALT, AST and ALP concentration at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. Therefore, in term of improvement in serum ALT, AST and ALP concentration in treated groups of buffaloes best recovery was assessed in Group A followed by Group B and minimum in Group C. In present study the elevated level of serum ALT, AST, ALP in the positive cases before treatment are found in agreement with the findings of Awobode (2006); Omejea *et al.* (2012). The causes of the elevation of ALT, AST levels may be due to necrosis of the liver, skeletal muscles and kidneys (Lording and Friend, 1991; Abeer *et al.*, 2011). The values of ALT, AST and ALP were found to be decreased after treatment with different drugs on the day 7<sup>th</sup> and day 14<sup>th</sup> post treatment in all the groups but the best recovery was observed in treatment with isometamidium followed by diminazene and quinapyramine.

There was a significant reduction ( $P<0.05$ ) in blood glucose concentration (at day, 0) in all the treatment groups (A, B and C) of buffaloes in comparison with the healthy control (Table ???). However, there was a significant increase ( $P<0.05$ ) in the blood glucose concentration at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in all treatment groups with highest recovery in Group A followed by Group B and minimum in Group C. Therefore, in terms of improvement in blood glucose concentrations in treated groups of buffaloes best recovery was assessed in Group A followed by Group B and minimum in Group C. Hypoglycemia has been reported in natural trypanosomiasis in human and animal by Moon *et al.* (1968), Wellde *et al.* (1974). Excessive utilization of the blood glucose by trypanosomes for their metabolism has been thought to account for the hypoglycemia observed

Table 1. Percent recovery assessment.

Groups	Percent recovery on day 7 <sup>th</sup>	Percent recovery on day 14 <sup>th</sup>
Group A	100	100
Group B	83.33	100
Group C	50	<100

Table 2A. Hematological alterations in various groups of buffaloes suffering from trypanosomiasis.

Groups	Hb (gm/dl)			TEC ( $\times 10^6/\mu\text{l}$ )			PCV (%)		
	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>
A	6.52 $\pm$ 0.31 <sup>Aa</sup>	8.68 $\pm$ 0.14 <sup>Cb</sup>	10.35 $\pm$ 0.24 <sup>Bc</sup>	4.37 $\pm$ 0.12 <sup>Aa</sup>	6.88 $\pm$ 0.11 <sup>Cb</sup>	7.58 $\pm$ 0.08 <sup>Cc</sup>	20.83 $\pm$ 0.79 <sup>Aa</sup>	27.5 $\pm$ 0.92 <sup>Bb</sup>	33.167 $\pm$ 1.08 <sup>Bc</sup>
B	6.55 $\pm$ 0.30 <sup>Aa</sup>	7.83 $\pm$ 0.04 <sup>Bb</sup>	9.77 $\pm$ 0.23 <sup>Bc</sup>	4.55 $\pm$ 0.06 <sup>Aa</sup>	5.33 $\pm$ 0.17 <sup>Bb</sup>	6.78 $\pm$ 0.16 <sup>Bc</sup>	20.67 $\pm$ 0.84 <sup>Aa</sup>	25.00 $\pm$ 0.45 <sup>Ab</sup>	32.83 $\pm$ 0.95 <sup>Bc</sup>
C	6.55 $\pm$ 0.34 <sup>Aa</sup>	7.40 $\pm$ 0.14 <sup>Ab</sup>	8.23 $\pm$ 0.08 <sup>Ac</sup>	4.40 $\pm$ 0.12 <sup>Aa</sup>	4.75 $\pm$ 0.07 <sup>Ab</sup>	5.65 $\pm$ 0.10 <sup>Ac</sup>	20.90 $\pm$ 0.96 <sup>Aa</sup>	23.30 $\pm$ 0.48 <sup>Ab</sup>	26.33 $\pm$ 0.49 <sup>Ac</sup>
D	10.77 $\pm$ 0.28 <sup>B</sup>	10.70 $\pm$ 0.18 <sup>D</sup>	10.90 $\pm$ 0.21 <sup>C</sup>	7.68 $\pm$ 0.09 <sup>B</sup>	7.85 $\pm$ 0.13 <sup>D</sup>	7.87 $\pm$ 0.14 <sup>C</sup>	32.17 $\pm$ 0.75 <sup>B</sup>	32.83 $\pm$ 0.65 <sup>C</sup>	33.17 $\pm$ 0.31 <sup>B</sup>

Table 2B. Hematological alterations in various groups of buffaloes suffering from trypanosomiasis.

Groups	TLC ( $\times 10^3/\mu\text{l}$ )			Neutro. (%)			Lympho. (%)		
	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>
A	13.15 $\pm$ 0.19 <sup>Be</sup>	10.27 $\pm$ 0.19 <sup>Bb</sup>	8.7 $\pm$ 0.20 <sup>Aa</sup>	70.17 $\pm$ 0.60 <sup>Be</sup>	32.75 $\pm$ 0.31 <sup>Bb</sup>	31.39 $\pm$ 0.56 <sup>Aa</sup>	24.59 $\pm$ 0.62 <sup>Aa</sup>	62.33 $\pm$ 0.56 <sup>Cb</sup>	62.83 $\pm$ 0.31 <sup>Bb</sup>
B	13.1 $\pm$ 0.20 <sup>Bc</sup>	12.57 $\pm$ 0.06 <sup>Cb</sup>	10.43 $\pm$ 0.12 <sup>Ba</sup>	68.40 $\pm$ 1.22 <sup>Bc</sup>	42.55 $\pm$ 0.70 <sup>Cb</sup>	32.08 $\pm$ 1.01 <sup>Aa</sup>	26 $\pm$ 1.55 <sup>Aa</sup>	52.5 $\pm$ 0.85 <sup>Bb</sup>	61.83 $\pm$ 0.60 <sup>Bc</sup>
C	13.03 $\pm$ 0.09 <sup>Be</sup>	12.47 $\pm$ 0.08 <sup>Cb</sup>	12.00 $\pm$ 0.12 <sup>Ca</sup>	70.06 $\pm$ 0.70 <sup>Bc</sup>	49.62 $\pm$ 0.87 <sup>Db</sup>	36.49 $\pm$ 1.34 <sup>Ba</sup>	24.00 $\pm$ 0.82 <sup>Aa</sup>	45.50 $\pm$ 0.99 <sup>Ab</sup>	57.50 $\pm$ 1.50 <sup>Ac</sup>
D	8.93 $\pm$ 0.14 <sup>A</sup>	8.77 $\pm$ 0.17 <sup>A</sup>	8.93 $\pm$ 0.22 <sup>A</sup>	32.17 $\pm$ 0.70 <sup>A</sup>	32.26 $\pm$ 0.75 <sup>A</sup>	32.50 $\pm$ 1.18 <sup>A</sup>	62.33 $\pm$ 0.33 <sup>B</sup>	62.33 $\pm$ 0.56 <sup>C</sup>	61.21 $\pm$ 0.49 <sup>B</sup>

Mean with different superscript (A, B, C, D) in columns are differing significantly in between the groups, otherwise non-significant. Mean with different superscript (a, b, c) in rows are differing significantly in between the intervals, otherwise non-significant.

Table 2C. Hematological alterations.

Groups	Mono. (%)			Baso (%)			Eosi (%)		
	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>
A	2.5±0.62	2.83±0.31	3.67±0.59	0.57±0.03	0.59±0.04	0.61±0.02	2.17±0.46	1.5±0.32	1.48±0.41
B	2.87±0.69	3.18±0.49	4.27±0.81	0.23±0.06	0.27±0.08	0.33±0.04	2.5±0.670	1.5±0.50	1.49±0.62
C	3.27±0.54	3.19±0.49	4.30±0.64	0.17±0.04	0.19±0.02	0.21±0.07	2.50±0.52	1.50±0.59	1.50±0.62
D	3.00±0.65	3.67±0.21	4.15±0.51	0.50±0.05	0.57±0.03	0.62±0.07	2.00±0.57	1.17±0.40	1.50±0.34

Mean with different superscript (A, B, C, D) in columns are differing significantly in between the groups, otherwise non-significant.

Mean with different superscript (a, b, c) in rows are differing significantly in between the intervals, otherwise non-significant

Table 3A. Biochemical alterations in various groups of buffaloes suffering from trypanosomiasis.

Groups	T. protein (gm/dl)			Albumin (gm/dl)			Globulin (gm/dl)		
	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>
A	7.00±0.07	7.07±0.06	7.08±0.08	1.67±0.05 <sup>Aa</sup>	2.68±0.09 <sup>Bb</sup>	3.00±0.07 <sup>Cc</sup>	5.33±0.10 <sup>Bc</sup>	4.39±0.07 <sup>Bb</sup>	4.08±0.05 <sup>Aa</sup>
B	6.93±0.07	7.00±0.03	7.03±0.05	1.50±0.11 <sup>Aa</sup>	2.08±0.07 <sup>Ab</sup>	2.85±0.06 <sup>Cc</sup>	5.43±0.11 <sup>Bc</sup>	4.92±0.05 <sup>Cb</sup>	4.8±0.06 <sup>Aa</sup>
C	6.92±0.08	6.97±0.07	7.00±0.12	1.70±0.04 <sup>Aa</sup>	1.94±0.05 <sup>Ab</sup>	2.45±0.06 <sup>Ac</sup>	5.22±0.10 <sup>Bc</sup>	5.03±0.08 <sup>Cb</sup>	4.55±0.09 <sup>Ba</sup>
D	7.05±0.09	7.07±0.09	7.15±0.10	2.90±0.07 <sup>B</sup>	2.97±0.06 <sup>C</sup>	2.88±0.07 <sup>C</sup>	4.15±0.08 <sup>A</sup>	4.10±0.07 <sup>A</sup>	4.27±0.12 <sup>A</sup>

Table 2C. Hematological alterations.

Groups	Blood glucose (mg/dl)			BUN (mg/dl)			Creatinine (mg/dl)		
	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>
A	27.60±1.42 <sup>Aa</sup>	45.5±0.85 <sup>Bb</sup>	50.00±1.06 <sup>Bc</sup>	27.83±0.79 <sup>Bc</sup>	20.17±0.48 <sup>Bb</sup>	12.8±0.62 <sup>Aa</sup>	1.98±0.15 <sup>Bb</sup>	1.50±0.07 <sup>Aa</sup>	1.50±0.07 <sup>a</sup>
B	27.50±1.40 <sup>Aa</sup>	32.83±0.65 <sup>Ab</sup>	48.83±1.51 <sup>Bc</sup>	27.67±0.72 <sup>Bc</sup>	24.17±0.48 <sup>Cb</sup>	13.83±0.70 <sup>Aa</sup>	2.41±0.12 <sup>Cb</sup>	1.88±0.15 <sup>Ba</sup>	1.70±0.14 <sup>a</sup>
C	27.52±1.41 <sup>Aa</sup>	30.30±1.08 <sup>Aa</sup>	38.33±0.92 <sup>Ab</sup>	28.00±0.77 <sup>Bc</sup>	25.33±0.56 <sup>Cb</sup>	21.00±0.37 <sup>Ba</sup>	2.01±0.14 <sup>B</sup>	2.00±0.15 <sup>B</sup>	1.72±0.15
D	49.83±0.79 <sup>B</sup>	50.17±0.91 <sup>C</sup>	50.00±0.77 <sup>B</sup>	13.17±0.48 <sup>A</sup>	13.17±0.40 <sup>A</sup>	13.67±0.49 <sup>A</sup>	1.48±0.15 <sup>A</sup>	1.43±0.10 <sup>A</sup>	1.58±0.12

Table 3C. Biochemical alterations in various groups of buffaloes suffering from trypanosomiasis.

Groups	ALT (IU/L)			AST (IU/L)			ALP (IU/L)		
	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>	Day 0	Day 7 <sup>th</sup>	Day 14 <sup>th</sup>
A	64.67±3.42 <sup>Bc</sup>	36.5±1.34 <sup>Bb</sup>	29.5±1.06 <sup>Aa</sup>	140.17±1.87 <sup>Bb</sup>	116.67±3.36 <sup>Aa</sup>	116.35±3.17 <sup>Aa</sup>	159.17±1.08 <sup>Bc</sup>	115.67±2.08 <sup>Bb</sup>	109.33±1.36 <sup>Aa</sup>
B	65.33±3.22 <sup>Bc</sup>	47.50±1.50 <sup>Cb</sup>	32.00±1.26 <sup>Aa</sup>	141.33±1.96 <sup>Bb</sup>	137.33±1.45 <sup>Bb</sup>	118.12±1.24 <sup>Aa</sup>	158.50±1.12 <sup>Bc</sup>	137.16±1.05 <sup>Cb</sup>	109.50±3.38 <sup>Aa</sup>
C	67.00±3.06 <sup>Bb</sup>	61.16±3.21 <sup>Db</sup>	40.83±1.35 <sup>Ba</sup>	142.33±1.91 <sup>B</sup>	139.33±2.33 <sup>B</sup>	135.67±2.23 <sup>B</sup>	159.67±1.61 <sup>Bc</sup>	146.83±1.42 <sup>Db</sup>	135.50±0.76 <sup>Ba</sup>
D	29.67±1.54 <sup>A</sup>	30.17±0.91 <sup>A</sup>	31.00±1.15 <sup>A</sup>	116.67±3.61 <sup>A</sup>	117.00±3.14 <sup>A</sup>	117.33±3.25 <sup>A</sup>	109.33±1.31 <sup>A</sup>	109.83±1.19 <sup>A</sup>	110.33±1.09 <sup>A</sup>

Mean with different superscript (A, B, C, D) in columns are differing significantly in between the groups, otherwise non-significant.

Mean with different superscript (a, b, c) in rows are differing significantly in between the intervals, otherwise non-significant.

during trypanosomiasis (Anosa, 1988a). Values of blood glucose were found to be significantly elevated after treatment with different drugs on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment in all the groups but best recovery observed in treatment with isometamedium followed by diaminazine and quinopyramine.

From present study it can be concluded that all three drugs were effective against the trypanosomiasis in buffalo but the extent of improvement in terms of hematological values, biochemical values and percent recovery was observed maximum in the treatment with isometamidium chloride hydrochloride followed by diminazene aceturate and least with the quinapyramine sulphate. Therefore, the order of comparative efficacy of three anti-trypanosomal drugs in the present study has been found as Isometamidium Chloride hydrochloride > Diminazene aceturate > Quinapyramine sulphate.

### ACKNOWLEDGEMENT

Authors are thankful to the Dean of the College of Veterinary Sciences, and Dean Post Graduate Studies, DUVASU, Mathura, for providing necessary facilities to carry out the study.

### REFERENCES

- Abeer, A., E.B. Abd and I.S. Shaymaa. 2011. Clinicopathological and cytological studies on naturally infected camels and experimentally infected rats with *Trypanosoma evansi*. *World Applied Sciences Journal*, **14**(1): 42-50. Available on: [https://www.idosi.org/wasj/wasj14\(1\)11/6.pdf](https://www.idosi.org/wasj/wasj14(1)11/6.pdf)
- Abenga, J.N. and V.O. Anosa. 2005. Serum total proteins and creatinine levels in experimental *Gambian trypanosomosis* of vervet monkeys. *Afr. J. Biotech*, **4**(2): 187-190. Available on: <https://tspace.library.utoronto.ca/bitstream/1807/6612/1/jb05035.pdf>
- Ajayi, O.O., E.L. Toshak, O.B. Obaloto, B. Iliyasu, A.C. Igweh, A.J. Dadah and C.O. Idehen. 2013. Comparative efficacy of Berenil and Samorin in albino rats experimentally infected with current field isolates of *T. brucei brucei*. *International Journal of Biological and Chemical Sciences*, **7**(4): 1452-1460. DOI: 10.4314/ijbcs.v7i4.3
- Akanji, M.A., O.S. Adeyemi, S.O. Oguntoye and F. Sulyman. 2009. *Psidium guajava* extract reduces trypanosomosis associated lipid peroxidation and raises glutathione concentrations in infected animals. *EXCLI J.*, **8**: 148-54. DOI: 10.17877/DE290R-8903
- Anene, B.M., A. Ifebigh, I.A. Igwilo and P.U. Umeakuana. 2011. Prevalence and haemato-biochemical parameters of trypanosomes infected pigs at Nsukka, Nigeria. *Comparative Clinical Pathology*, **20**(1): 15-18. DOI: 10.1007/s00580-009-0944-2
- Anosa, V.O. 1988a. Haematological; and biochemical changes in human and animal trypanosomosis, Part I. *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux*, **4**(1): 65-78.
- Anosa, V.O. 1988b. Haematological; and biochemical changes in human and animal trypanosomosis, Part II. *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux*, **4**(2): 151-164.

- Awa, D.N. and C.N. Ndamkou. 2006. Response of *Trypanosoma vivax* and *Trypanosoma congolense* in zebu cattle in north Cameroon to prophylactic treatment with two formulations of isometamidium. *Prev. Vet. Med.*, **76**(1-2): 90-96. DOI: 10.1016/j.prevetmed.2006.04.011
- Awobode, H.O. 2006. The biochemical changes induced by natural human African trypanosome infections. *Afr. J. Biotechnol.*, **5**(9): 738-742.
- Baron, D.N., J.T. Whichen and K.E. Lee. 1989. *A New Short Textbook of Chemical Pathology*, 5<sup>th</sup> ed. (ELBS) Butler and Tanner Ltd., London Educational, Academic and Medical Publication Division of Hodder and Stoughton London, UK.
- Coles, E.H. 1986. *Veterinary Clinical Pathology*, 4<sup>th</sup> ed. W.B.S. Saunder Co. Philadelphia, USA.
- Dasilva, A.S., R.A. Zanette, P. Wolkmer, M.M. Costa, H.A. Garcia, S.T.A. Lopes, J.M. Santurio, M.M.G. Teixeira and S.G. Monteiro. 2009. Diminazene aceturate in the control of *T. evansi* infection in cats. *Vet. Parasitol.*, **165**(1-2): 47-50. DOI: 10.1016/j.vetpar.2009.06.025
- Gill, B.S. 1991. *Trypanosomes and Trypanosomiasis in Indian Livestock*. Indian Council of Agricultural Research Publication, New Delhi, India.
- Herbert, W.J. and M.D. Inglis. 1973. Immunization of mice, against *T. brucei* infection, by the administration of released antigen adsorbed to erythrocytes. *Royal Society of Tropical Medicine and Hygiene*, **67**(2): 268. DOI: 10.1016/0035-9203(73)90174-0
- Hilali, M., G.A. Abdel, A. Nassar and W.A. Abdel. 2006. Hematological and biochemical changes in water buffalo calves (*B. bubalis*) infected with *T. evansi*. *Vet. Parasitol.*, **139**(1-3): 237-243. DOI: 10.1016/j.vetpar.2006.02.013
- Howes, F., A.S. Da Silva, C. de L. Athayde, M.M. Costa, M.M.B. Corrêa, K.C.S. Tavares, L.C. Miletti, S.T. dos A. Lopes, A.S. do Amaral and C. Schmidt. 2011. A new therapeutic protocol for dogs infected with *T. evansi*. *Acta Sci. Vet.*, **39**(3): 988. Available on: <http://www.ufrgs.br/actavet/39-3/PUB%20988.pdf>
- Kadima, K.B., O.G. Erastus, I.S. Daniel and A.N.E. King. 2000. Serum biochemical values of *T. vivax*-infected cattle and the effects of lactose in saline infusion. *Vet. Archiv.*, **70**(2): 67-74.
- Keniko, J.J. 1997. *Clinical Biochemistry of Domestic Animals*, 5<sup>th</sup> ed. Academic Press, San Diego, USA.
- Kennedy, G.E.P. 2004. Human African trypanosomiasis of the CNS: Current issues and challenges. *J. Clin. Invest.*, **13**(4): 496-504. DOI: 10.1172/JCI21052
- Kulkarni, M.D., M.B. Nisal, R. Joshin and P.E. Kulkarni. 1997. Haemodynamic studies of trypanosomiasis in buffaloes (*Bulalus bubalis*). *Indian Vet. J.*, **74**(11): 981-982.
- Kulkarni, M.D., M.B. Nisal, J.M.K. Rao and P.E. Kulkarni. 1996. Surra in cattle. *Livestock Adviser*, **21**: 3-4.
- Kumar, H., M.P. Gupta, P.K. Sidhu, V. Mahajan, M.S. Bal, K. Kaur, A.S. Verma and L.D. Singla. 2012. An outbreak of acute *T. evansi* infection in crossbred cattle in Punjab, India. *Journal of Applied Animal Research*, **40**(3): 256-259. DOI: 10.1080/09712119.2012.667651
- Kumar, R.M., N.M. Kamili, A. Saxena, A. Dan,

- S. Dey and S.S. Raut. 2011. Disturbance of oxidant/antioxidant equilibrium in horses naturally infected with *T. evansi*. Elsevier, *Vet. Parasitol.*, **180**(3-4): 349-353. DOI: 10.1016/j.vetpar.2011.03.029
- Kumar, U., R. Jas and J.D. Ghosh. 2009. Effect of isometamidium hydrochloride on *T. evansi* infections in rats. *Journal of Parasitic Diseases*, **33**(1-2): 36-41. DOI: 10.1007/s12639-009-0006-3
- Lording, P.M. and S.C.E. Friend. 1991. Data analysis guide. Interpretation of laboratory results. *Aust. Vet. Pract.*, **21**: 186-195.
- Monzon, C.M., O.A. Mancebo and J.P. Roux. 1990. Comparison between six parasitological methods for diagnosis of *T. evansi* in the subtropical area of Argentina. *Vet. Parasitol.*, **36**(1-2): 141-146. DOI: 10.1016/0304-4017(90)90102-H
- Moon, A.P., J.S. Williams and C. Witherspoon. 1968. Serum biochemical changes in mice infected with *T. rhodesiense* and *T. duttoni*. *Experimental Parasitology*, **22**(1): 112-121. DOI: 10.1016/0014-4894(68)90084-2
- Omejea, J.N. and B.M. Aneneb. 2012. Comparative serum biochemical changes induced by experimental infection of *T. brucei* and *T. congolense* in pigs. *Vet. Parasitol.*, **190**(2-4): 368-374. DOI: 10.1016/j.vetpar.2012.07.008
- Pathak, K.M.L. and N. Singh. 2005. Animal trypanosomiasis. *Intas Polivet*, **6**(11): 194-199.
- Pholpark, S., M. Pholpark, S. Sarataphan, S. Khunpasi and P. Taboran. 1984. *T. evansi* infection in buffalo in northeast Thailand. In *Proceedings of the 11<sup>th</sup> Annual Conference of the Thailand Veterinary Medical Association*, Bangkok, Thailand.
- Taiwo, V.O., M.O. Olaniyi and A.O. Ogunsanmi. 2003. Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental *T. congolense* and *T. brucei* infections in sheep. *Isr. J. Vet. Med.*, **58**(4): 30-33.
- Takeet, M. and B.O. Fagbemi. 2009. Haematological, pathological and plasma biochemical changes in rabbits experimentally infected with *T. congolense*. *Science World Journal*, **4**(2): 29-36. DOI: 10.4314/swj.v4i2.51843
- Toro, M., E. León, R. López, F. Pallota and A. Ruiz. 1983. Effect of isometamidium on infections by *T. vivax* and *T. Evansi* in experimentally infected animals. *Vet. Parasitol.*, **13**(1): 35-43. DOI: 10.1016/0304-4017(83)90018-3
- Ugochukwu, E.I. 1986. Haematological observations on bovine trypanosomiasis of Holstein-Friesian breed. *Int. J. Zoonoses*, **13**(2): 89-92.
- Walia, P.S., I.S. Kalra, P.D. Juyal and S.P. Ahuja. 1996. Role of activity of *Trypanosoma evansi* in inducing anemia and immunomodulation in buffalo calves. *J. Vet. Parasitol.*, **10**(1): 1-9.
- Wellde, B.T., R. Lotzsch, G. Diehl, E. Sadun, J. Williams and G. Warui. 1974. *Trypanosoma congolense*: I. clinical observations of experimentally infected cattle. *Exp. Parasitol.*, **36**(1): 6-19. DOI: 10.1016/0014-4894(74)90107-6
- Wilkinson, J.H. 1962. *An Introduction to Diagnostic Enzymology*. Edward Arnold (Publishers) Ltd., London, UK. p. 1-277.