



*Original Research*

## Assessment of Clinical Recovery of Equine Trypanosomosis using Antitrypanosomal Drugs and Antioxidant Supportive Therapy

R. K. Singh, A. K. Tripathi\*, A. Srivastava and A. P. Singh

Department of Veterinary Medicine, Pt. Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya evam Go Anusandhan Sansthan (DUVASU), Mathura- 281001, Uttar Pradesh, INDIA

\*Corresponding author: [arvindvet04@rediffmail.com](mailto:arvindvet04@rediffmail.com)

|              |   |
|--------------|---|
| Rec. Date:   | Sep 27, 2018 06:06  |
| Accept Date: | Feb 22, 2019 13:36  |
| DOI          | <a href="https://doi.org/10.5455/ijlr.20180927060618">10.5455/ijlr.20180927060618</a> |

### Abstract

*The therapeutic efficacy of various antitrypanosomal drugs viz. diminazine aceturate, isometamidium chloride HCl and quinopyramine sulphate alone or in combination with vitamin E (antioxidant) were evaluated in naturally infected equines with T. evansi. Evaluation was done on the basis of alterations in the hemato-biochemical, oxidative stress parameters and percent recovery assessment on day 0 pretreatments, day 7<sup>th</sup> and day 14<sup>th</sup> post treatment. It can be infer from present study that all three drugs alone or along with vitamin E were effective against the trypanosomosis in equines but the extent of improvement in terms of hemato-biochemical values, oxidative stress parameters and percent recovery was observed maximum in the treatment with diminazine aceturate with vitamin E followed by isometamidium chloride hydrochloride with vitamin E and least with the quinopyramine sulphate. Therefore, the order of comparative therapeutic efficacy of anti-trypanosomal drugs in the present study has been found as diminazene aceturate with vitamin E, maximally effective followed by isometamidium chloride HCL with vitamin E and Quinopyramine sulphate was found to be least effective. Hence, diminazine aceturate along with vitamin E (antioxidant) could be successfully used in the treatment of equine trypanosomosis.*

**Key words:** Equine, Hemato-Biochemical, Oxidative Stress, Percent Recovery, Therapeutic Efficacy, Trypanosomosis

**How to cite:** Singh, R., Tripathi, A., Srivastava, A., & Singh, A. (2019). Assessment of Clinical Recovery of Equine Trypanosomosis using Antitrypanosomal Drugs and Antioxidant Supportive Therapy. International Journal of Livestock Research, 9(4), 94-102. doi: 10.5455/ijlr.20180927060618

### Introduction

Majority of the equines (97.96%) in India are owned by landless, small and marginal farmers belonging to socio-economically deprived communities in rural and semi-urban areas is mostly unorganized. Surra mostly occurs as an endemic disease in northern and eastern India and treated horses have a high risk of re-infection (Laha and Sasmal, 2008). The field control of animal trypanosomiasis has, over the years, relies



on two broad strategies: using chemotherapeutic agents on infected animals and vector control. In general, however, the chemotherapeutic approach is used much more widely than vector control because it is easier to kill the trypanosomes than the flies (WHO 1998). Current methods of treatment of trypanosomes are still unsatisfactory usually associated with severe side effects and indiscriminate use of anti-trypanosomal drugs results into the emergence of drug resistance (Kaminsky and Brun, 1998). Keeping in view of the above facts this study was done to compare *in vivo* efficacy of the three commonly used chemotherapeutic drugs alone and in combination with the vitamin E (Diminazene aceturate, Quinapyramine sulphate and isometamidium chloride HCl) on equines clinically infected with *T. evansi*.

### Materials and Methods

The study was performed at teaching veterinary clinical complex (TVCC), Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura (DUVASU) from April, 2014 to March, 2015. The comparative efficacies of three anti-trypanosomal drugs (diminazine aceturate, quinapyramine sulphate and isometamidium chloride hydrochloride) alone and along with vitamin-E as antioxidants were used in standard dosage in positive cases of *Trypanosoma evansi* by giemsa stained thin blood smear. Prior to administration of antitrypanosomal drugs, animals were administered supportive therapies. These animals were grouped in seven groups with six equines in each groups (n=6).

|           |  |
|-----------|--|
| Group I   | Positive cases of <i>Trypanosoma evansi</i> treated by diminazene aceturate (@ 7mg/kg body wt. by deep I/M rout) along with supportive therapy.  |
| Group II  | Positive cases of <i>Trypanosoma evansi</i> treated by diminazene aceturate (@ 7mg/kg body wt. by deep I/M route) with Vitamin- E (@ 6 IU/kg body wt. orally for 2weeks) as antioxidant along with supportive therapy.         |
| Group III | Positive cases of <i>Trypanosoma evansi</i> treated by quinapyramine sulphate (@ 5mg/kg body wt. by S /C route) along with supportive therapy  |
| Group IV  | Positive cases of <i>Trypanosoma evansi</i> treated by quinapyramine sulfate (@ 5mg/kg body wt. by S /C route) with Vitamin -E (@ 6 IU/kg body wt. orally for 2weeks) as antioxidant along with supportive therapy.            |
| Group V   | Positive cases of <i>Trypanosoma evansi</i> treated by isometamidium Chloride HCl (@ 0.5 mg/kg body wt. by deep I/M route) along with supportive therapy.  |
| Group VI  | Positive cases of <i>Trypanosoma evansi</i> treated by isometamidium Chloride HCl (@ 0.5 mg/kg body wt. by deep I/M route) with Vitamin-E (@ 6 IU/kg body wt. orally for 2weeks) as antioxidant along with supportive therapy. |
| Group VII | Apparently healthy equine were kept as control   |

Supportive therapy was done as per need of the animal like fluid therapy, antipyretics, haematinics, antibiotics, antihistaminics etc. The therapeutic efficacy of above anti-trypanosomal drugs were evaluated on the basis of alterations in the hemato-biochemical, oxidative stress parameters and percent recovery assessment on day 0 pre-treatment, day 7<sup>th</sup> and 14<sup>th</sup> post treatment. All the positive cases of each group

were thoroughly examined by clinical examination and blood smear examination on the day 0 (pretreatment), 7<sup>th</sup> and 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 equines on the day 7<sup>th</sup> and 14<sup>th</sup> after treatment ( $\% \text{ Recovery} = n/6 \times 100$ , where n= number of blood smear negative and clinically improved equines). 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. Hemato-biochemical and oxidative stress parameter analysis were done on the day 0 (pretreatment), 7<sup>th</sup> and 14<sup>th</sup> (post-treatment). To assess the therapeutic efficacy of different anti-trypanosomal drugs, parameters *viz* hemoglobin, TEC, PCV, TLC, and DLC were assessed using hematology auto analyzer (Diatron's Abacus Hematology Analyzer, Wien, Australia).

For biochemical study serum was separated and estimation of total protein (Biuret method), albumin (BCG Dye method), globulin (Total protein-albumin), creatinine (Jaff's method), blood urea nitrogen (NED-dye method) and ALT by Modified UV (IFCC) with the help of BS-120 Chemistry Analyzer (Shenzhen Mindray Biochemical Electronics Co. Ltd.) using Span diagnostic kits (Span Diagnostics Ltd, Sachin, Surat, India). Estimation of glucose was done on the spot of sample collection by using glucometer (Gluco Chek, Aspen Diagnostics (P) LTD. Delhi-33, India). To evaluate the oxidative stress parameters the extent of lipid peroxidation was evaluated in terms of MDA (malondialdehyde) production, determined by thiobarbituric acid (TBA) method. Superoxide dismutase (SOD) and catalase activity was estimated in the RBC hemolysate as per standard procedure. The data generated from the present study was subjected to the test of significance (t-test) as per the method described (Thrusfield, 2008).

## Results and Discussion

### Therapeutic Efficacy on the Basis of Hemato-Biochemical Alterations

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 of equines in comparison with the healthy control (Table 1). 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 II followed by group VI and minimum in group III. Therefore in terms of improvement in treated groups of equines best recovery was assessed in treatment with diminazene aceturate with vitamin E, followed by isometamidium chloride hydrochloride with vitamin-E and least with quinopyramine sulphate. The present findings of decrease in Hb, PCV and TEC in cases of trypanosomiasis are in agreement with the findings of Kumar *et al.* (2011). The total leukocyte count, neutrophils and lymphocytes were significantly higher ( $p < 0.05$ ) (at day, 0) in all the treatment groups of equines in comparison with the healthy control.

**Table 1:** Hematological alterations in various groups of equines suffering from *Trypanosoma evansi* infection (Mean ± SE)

| Groups     | Hb (gm/dl)               |                           |                            | TEC (×10 <sup>6</sup> /μl) |                          |                                      | 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>                |
| <b>I</b>   | 6.78 ±0.14 <sup>Aa</sup> | 10.80 ±0.06 <sup>Db</sup> | 12.50 ±0.15 <sup>Cc</sup>  | 4.02 ±0.02 <sup>Aa</sup>   | 6.89 ±0.02 <sup>Cb</sup> | 7.04 ±0.02 <sup>BCc</sup>            | 20.00 ±0.51 <sup>Aca</sup> | 32.78 ±0.14 <sup>Db</sup> | 34.5±0.48 <sup>A<sup>B</sup>c</sup> |
| <b>II</b>  | 6.85±0.08 <sup>Aa</sup>  | 11.95 ±0.21 <sup>Eb</sup> | 13.47 ±0.09 <sup>Dc</sup>  | 4.16±0.08 <sup>Aa</sup>    | 7.35 ±0.12 <sup>Db</sup> | 7.85 ±0.10 <sup>Dc</sup>             | 20.45 ±0.30 <sup>Aa</sup>  | 35.65±0.70 <sup>Eb</sup>  | 39.56±0.23 <sup>Ec</sup>            |
| <b>III</b> | 6.78 ±0.09 <sup>Aa</sup> | 7.55 ±0.11 <sup>Ab</sup>  | 11.53 ±0.084 <sup>Ac</sup> | 4.24±0.10 <sup>Aa</sup>    | 5.47 ±0.13 <sup>Ab</sup> | 6.65 ±0.15 <sup>Ac</sup>             | 20.63 ±0.32 <sup>Aa</sup>  | 22.33±0.20 <sup>Ab</sup>  | 34.27±0.18 <sup>Ac</sup>            |
| <b>IV</b>  | 6.78 ±0.06 <sup>Aa</sup> | 7.95 ±0.11 <sup>Bb</sup>  | 11.87 ±0.05 <sup>Bc</sup>  | 4.15±0.08 <sup>Aa</sup>    | 5.70 ±0.12 <sup>Ab</sup> | 6.90 ±0.12 <sup>ABc</sup>            | 20.40 ±0.24 <sup>Aa</sup>  | 23.72±0.42 <sup>Bb</sup>  | 35.5±0.16 <sup>B<sup>C</sup>c</sup> |
| <b>V</b>   | 6.90 ±0.12 <sup>Aa</sup> | 8.03 ±0.10 <sup>Bb</sup>  | 12±0.11 <sup>Bc</sup>      | 4.22±0.10 <sup>Aa</sup>    | 5.78 ±0.10 <sup>Ab</sup> | 7.17 ±0.10 <sup>BCc</sup>            | 20.80 ±0.48 <sup>Aa</sup>  | 24.13±0.28 <sup>Bb</sup>  | 36.53±0.62 <sup>CDc</sup>           |
| <b>VI</b>  | 6.88 ±0.19 <sup>Aa</sup> | 9.13 ±0.13 <sup>Cb</sup>  | 12.47±0.147 <sup>Cc</sup>  | 4.07±0.06 <sup>Aa</sup>    | 6.3 ±0.12 <sup>Bb</sup>  | 7.28 ±0.10 <sup>Cc</sup>             | 20.17 ±0.30 <sup>Aa</sup>  | 27.38±0.43 <sup>Cb</sup>  | 37.38±0.41 <sup>Dc</sup>            |
| <b>VII</b> | 13.80 ±0.06 <sup>B</sup> | 14.00 ±0.07 <sup>F</sup>  | 14.05±0.08 <sup>E</sup>    | 8.83±0.05 <sup>B</sup>     | 8.87±0.08 <sup>E</sup>   | 9.00 ±0.07 <sup>E</sup>              | 40.13 ±0.41 <sup>B</sup>   | 40.93±0.39 <sup>F</sup>   | 41.38±0.32 <sup>F</sup>             |
| Groups     | TLC(×103/μl)             |                           |                            | Neutrophils (%)            |                          |                                      | Lymphocytes (%)            |                           |                                     |
|            | 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>                |
| <b>I</b>   | 16.45±0.13 <sup>Bb</sup> | 13.02±0.06 <sup>Ba</sup>  | 12.75±0.08 <sup>Ba</sup>   | 80.5±0.76 <sup>Bb</sup>    | 69±0.36 <sup>Ba</sup>    | 67.5±0.56 <sup>Da</sup>              | 15.67±0.49 <sup>Aa</sup>   | 26±1.13 <sup>Bb</sup>     | 28.67±1.14 <sup>Ab</sup>            |
| <b>II</b>  | 15.80±0.62 <sup>Bb</sup> | 12.95±0.06 <sup>Bav</sup> | 12.67±0.07 <sup>Ba</sup>   | 80.5±0.50 <sup>Bc</sup>    | 69±0.52 <sup>Bb</sup>    | 67.33±0.49 <sup>CDa</sup>            | 15.33±0.33 <sup>Aa</sup>   | 26.5±0.85 <sup>Bb</sup>   | 28.33±0.92 <sup>Ab</sup>            |
| <b>III</b> | 16.38±0.16 <sup>Bc</sup> | 15.45±0.15 <sup>Cb</sup>  | 13.77±0.14 <sup>Ca</sup>   | 79.67±0.84 <sup>Bc</sup>   | 75.5±0.85 <sup>Cb</sup>  | 68.17±0.54 <sup>Da</sup>             | 15±0.63 <sup>Aa</sup>      | 19.17±0.87 <sup>Ab</sup>  | 26.17±1.01 <sup>Ac</sup>            |
| <b>IV</b>  | 16.27±0.14 <sup>Bc</sup> | 15.25±0.16 <sup>Cb</sup>  | 13.67±0.05 <sup>Ca</sup>   | 79.33±0.84 <sup>Bc</sup>   | 75.17±1.01 <sup>Cb</sup> | 67.83±0.75 <sup>Da</sup>             | 15.33±0.42 <sup>Aa</sup>   | 19.83±1.11 <sup>Ab</sup>  | 26.67±0.76 <sup>Ac</sup>            |
| <b>V</b>   | 16.40±0.12 <sup>Bc</sup> | 15.3±0.12 <sup>Cb</sup>   | 13.63±0.12 <sup>Ca</sup>   | 80.83±0.70 <sup>Bc</sup>   | 74.17±0.60 <sup>Cb</sup> | 65.17±0.31 <sup>Ba</sup>             | 15±0.52 <sup>Aa</sup>      | 20±0.45 <sup>Ab</sup>     | 28.67±0.65 <sup>Ac</sup>            |
| <b>VI</b>  | 16.33±0.13 <sup>Bc</sup> | 15.28±0.10 <sup>Cb</sup>  | 13.6±0.13 <sup>Ca</sup>    | 81.33±0.72 <sup>Bc</sup>   | 73.5±0.76 <sup>Cb</sup>  | 65.83±0.48 <sup>B<sup>C</sup>a</sup> | 14±0.45 <sup>Aa</sup>      | 20.67±0.88 <sup>Ab</sup>  | 28±0.82 <sup>Ac</sup>               |
| <b>VII</b> | 11.08±0.06 <sup>A</sup>  | 11.25±0.08 <sup>A</sup>   | 11.21±0.07 <sup>A</sup>    | 56.17±0.48 <sup>A</sup>    | 54.5±0.50 <sup>A</sup>   | 54.67±0.42 <sup>A</sup>              | 38.83±0.79 <sup>B</sup>    | 40.5±0.22 <sup>C</sup>    | 40±0.36 <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.

There was a significant decrease ( $p < 0.05$ ) in the total leukocyte count, percent neutrophils and significant increase ( $p < 0.05$ ) in the lymphocytes 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 II followed by group VI and minimum in group III. 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. Leukocytosis and lymphocytopenia found in our study are in agreement with the findings of Abeer *et al.* (2011). Therefore, in term of improvement in total leukocyte count, neutrophils and lymphocyte percentage in treated groups of equines, best recovery was assessed in treatment with diminazene aceturate with vitamin E group, followed by Isometamidium chloride hydrochloride with vitamin E group and quinopyramine sulphate group.

Significant reduction ( $p < 0.05$ ) in the serum albumin concentration (at day, 0) was observed in all the treatment groups of equines in comparison with the healthy control (Table 2). 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 II followed by group I & VI and minimum in group III. There was a significant increase ( $p < 0.05$ ) in globulin concentration (at day, 0) in all the treatment groups of equines 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 II followed and minimum in group III. These findings of present investigations are in accordance with findings of Hilali *et al.* (2006). 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 was assessed in Group II followed by group VI and minimum in group III.

There was a significant reduction ( $p < 0.05$ ) in blood glucose concentration (at day, 0) in all the treatment groups of equines in comparison with the healthy control (Table 2). 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 I & II followed by group V & VI and minimum in group III. Our findings of hypoglycemia might have occurred due to the increase of the metabolic rate caused by fever has been reported in infected animals, hepatocyte degeneration or glucose consumption by the trypanosomes for their metabolism (Silva *et al.*, 1997). Therefore, in terms of improvement in blood glucose concentrations in treated groups of equines best recovery was assessed in Group I & II followed by group V & VI and minimum in group III. There was a significant increase ( $p < 0.05$ ) in serum blood urea nitrogen and serum creatinine concentration (at day, 0) in all the treatment groups of equines in comparison with the healthy control.

**Table 2:** Biochemical alterations in various groups of equines suffering from *Trypanosoma evansi* infection (Mean ± SE)

| Groups | Total 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>     |
| I      | 7.27±0.02                 | 7.32±0.03                | 7.37±0.02                 | 1.80±0.07 <sup>Ba</sup>  | 3±0.04 <sup>Cb</sup>                | 3.22±0.05 <sup>Cc</sup>  | 5.46±0.07 <sup>Bb</sup> | 4.32±0.06 <sup>Ba</sup>            | 4.15±0.05 <sup>Aa</sup>  |
| II     | 7.32±0.03                 | 7.38±0.05                | 7.48±0.03                 | 1.78±0.09 <sup>Ba</sup>  | 3.02±0.08 <sup>Cb</sup>             | 3.27±0.06 <sup>Cc</sup>  | 5.53±0.11 <sup>Bb</sup> | 4.37±0.09 <sup>Ba</sup>            | 4.21±0.07 <sup>Aa</sup>  |
| III    | 7.38±0.03                 | 7.55±0.04                | 7.62±0.03                 | 1.59±0.02 <sup>Aa</sup>  | 2.26±0.06 <sup>Ab</sup>             | 2.87±0.02 <sup>Ac</sup>  | 5.79±0.03 <sup>Cc</sup> | 5.29±0.08 <sup>Eb</sup>            | 4.75±0.04 <sup>Ca</sup>  |
| IV     | 7.4±0.04                  | 7.45±0.03                | 7.5±0.03                  | 1.56±0.04 <sup>Aa</sup>  | 2.25±0.06 <sup>Ab</sup>             | 2.89±0.02 <sup>Ac</sup>  | 5.84±0.06 <sup>Cc</sup> | 5.2±0.08 <sup>D<sup>Eb</sup></sup> | 4.59±0.05 <sup>BCa</sup> |
| V      | 7.4±0.04                  | 7.5±0.04                 | 7.57±0.04                 | 1.66±0.02 <sup>ABa</sup> | 2.56±0.01 <sup>Bb</sup>             | 3.03±0.02 <sup>Bc</sup>  | 5.74±0.04 <sup>Cc</sup> | 4.95±0.03 <sup>Cb</sup>            | 4.54±0.04 <sup>Ba</sup>  |
| VI     | 7.38±0.03                 | 7.53±0.02                | 7.62±0.04                 | 1.61±0.05 <sup>Aa</sup>  | 2.53±0.04 <sup>Bb</sup>             | 3.01±0.05 <sup>Bc</sup>  | 5.78±0.08 <sup>Cc</sup> | 5.01±0.04 <sup>CDb</sup>           | 4.6±0.07 <sup>BCa</sup>  |
| VII    | 7.25±0.06                 | 7.32±0.05                | 7.35±0.06                 | 3.15±0.05 <sup>C</sup>   | 3.18±0.06 <sup>D</sup>              | 3.3±0.04 <sup>C</sup>    | 4.17±0.08 <sup>A</sup>  | 4.07±0.09 <sup>A</sup>             | 4.05±0.08 <sup>A</sup>   |
| 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>     |
| I      | 53.33 ±1.02 <sup>Aa</sup> | 77.67±0.99 <sup>Cb</sup> | 83.17±1.62 <sup>Bc</sup>  | 58.39±0.37 <sup>Bb</sup> | 23.22±0.36 <sup>Ba</sup>            | 22.37±0.26 <sup>Ba</sup> | 2.27±0.05 <sup>Cc</sup> | 1.43±0.03 <sup>Cb</sup>            | 1.12±0.04 <sup>Aa</sup>  |
| II     | 53.17±0.87 <sup>Aa</sup>  | 77.17±1.01 <sup>Cb</sup> | 82.67±1.52 <sup>Bc</sup>  | 58.41±0.58 <sup>Bb</sup> | 22.95±0.28 <sup>Ba</sup>            | 22.2±0.29 <sup>Ba</sup>  | 2.06±0.08 <sup>Bc</sup> | 1.28±0.03 <sup>Bb</sup>            | 1.02±0.05 <sup>Aa</sup>  |
| III    | 53.67±0.84 <sup>Aa</sup>  | 64.17±0.70 <sup>Ab</sup> | 74.17±0.48 <sup>Ac</sup>  | 58.01±0.67 <sup>Bc</sup> | 32.88±0.28 <sup>Eb</sup>            | 24.04±0.05 <sup>Da</sup> | 2.63±0.05 <sup>Dc</sup> | 2.25±0.04 <sup>Db</sup>            | 1.97±0.01 <sup>Ca</sup>  |
| IV     | 53.83±0.95 <sup>Aa</sup>  | 63.5±0.72 <sup>Ab</sup>  | 74±0.58 <sup>Ac</sup>     | 57.87±0.57 <sup>Bc</sup> | 32.6±0.29 <sup>D<sup>Eb</sup></sup> | 23.97±0.06 <sup>Da</sup> | 2.57±0.07 <sup>Dc</sup> | 2.23±0.05 <sup>Db</sup>            | 1.96±0.01 <sup>Ca</sup>  |
| V      | 53.17±0.87 <sup>Aa</sup>  | 67.67±0.42 <sup>Bb</sup> | 76.83±0.48 <sup>Ac</sup>  | 58.2±0.44 <sup>Bc</sup>  | 31.7±0.23 <sup>CDb</sup>            | 23.19±0.23 <sup>Ca</sup> | 2.66±0.04 <sup>Dc</sup> | 2.35±0.07 <sup>Db</sup>            | 1.71±0.12 <sup>Ba</sup>  |
| VI     | 53±0.86 <sup>Aa</sup>     | 67.33±0.42 <sup>Bb</sup> | 76.5±0.56 <sup>Ac</sup>   | 57.88±0.39 <sup>Bc</sup> | 31.57±0.20 <sup>Cb</sup>            | 23.13±0.21 <sup>Ca</sup> | 2.66±0.05 <sup>Db</sup> | 2.39±0.08 <sup>Db</sup>            | 1.58±0.14 <sup>Ba</sup>  |
| VII    | 88±0.63 <sup>B</sup>      | 88.83±0.87 <sup>D</sup>  | 89±0.52 <sup>C</sup>      | 18.83±0.40 <sup>A</sup>  | 18.17±0.48 <sup>A</sup>             | 19.17±0.17 <sup>A</sup>  | 1.08±0.03 <sup>A</sup>  | 1.02±0.05 <sup>A</sup>             | 1±0.04 <sup>A</sup>      |
| 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>     |
| I      | 60.17±0.48 <sup>Bc</sup>  | 22.5±0.22 <sup>Bb</sup>  | 21±0.36 <sup>Ba</sup>     | 218.5±0.43               | 218.78±0.57                         | 218.43 ±0.53             | 185.67±0.49             | 186.33±0.67                        | 185.83±0.60              |
| II     | 60±0.63 <sup>Bb</sup>     | 22±0.36 <sup>Ba</sup>    | 20.83±0.31 <sup>Ba</sup>  | 217.67±0.33              | 217.98±0.30                         | 217.77 ±0.60             | 185.67±0.48             | 185.67±0.67                        | 185±0.45                 |
| III    | 58.5±0.76 <sup>Bc</sup>   | 32±0.58 <sup>Db</sup>    | 23.5±0.22 <sup>Da</sup>   | 219±0.45                 | 218.17±0.54                         | 217.33 ±0.72             | 186.17±0.60             | 185.83±0.48                        | 185.17±0.31              |
| IV     | 58.17±0.60 <sup>Bc</sup>  | 31.17±0.60 <sup>Db</sup> | 23.17±0.31 <sup>CDa</sup> | 217.83±0.70              | 218.33±0.49                         | 218.17 ±0.91             | 186±0.58                | 185.67±0.56                        | 185.67±0.33              |
| V      | 59±0.58 <sup>Bc</sup>     | 27.5±0.43 <sup>Cb</sup>  | 22.33±0.33 <sup>Ca</sup>  | 218.17±0.60              | 217.83±0.70                         | 217±0.58                 | 185.5±0.62              | 185.67±0.56                        | 186±0.36                 |
| VI     | 58.67±0.80 <sup>Bc</sup>  | 27.5±0.43 <sup>Cb</sup>  | 22.83±0.40 <sup>CDa</sup> | 217.5±0.67               | 217.33±0.76                         | 217.33 ±0.49             | 185±0.68                | 185.17±0.40                        | 185.67±0.88              |
| VII    | 20.17±0.70 <sup>A</sup>   | 19.33±0.49 <sup>A</sup>  | 19.5±0.22 <sup>A</sup>    | 219±0.36                 | 218.5±0.62                          | 218.50 ±0.50             | 180.17±0.70             | 179.5±0.56                         | 179.83±0.48              |

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-significa

However, there was a significant decrease ( $p < 0.05$ ) in serum blood urea nitrogen and serum creatinine after the treatment in all treatment groups with highest recovery in group II followed by group I & VI and minimum in group III. These elevations suggest renal injury and associated glomerular dysfunction (Anosa, 1988a). Therefore, in term of improvement in blood urea nitrogen and serum creatinine concentration in treated groups of equines best recovery was assessed in Group II followed by group I & VI and minimum in group III. There was a significant increase ( $p < 0.05$ ) in serum ALT concentration (at day, 0) in all the treatment groups of equines in comparison with the healthy control (Table 2). However, there was a significant decrease ( $p < 0.05$ ) in the serum ALT 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 II followed by group I & VI and minimum in group III. The causes of the elevation of ALT levels may be due to necrosis of the liver, skeletal muscles and kidneys (Abeer *et al.*, 2011). Therefore, in term of improvement in serum ALT concentration in treated groups of equines best recovery was assessed in Group II followed by group I & VI and minimum in group III.

#### **Therapeutic Efficacy on the Basis of Oxidative Stress Parameters**

There was a significant increase ( $p < 0.05$ ) in level of LPO concentration and decrease in SOD and catalase concentration in erythrocyte hemolysate (at day, 0) in all the treatment groups of equines in comparison with the healthy control (Table 3). However, there was a significant decrease ( $p < 0.05$ ) in the level of LPO concentration and increase in SOD and catalase 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 II followed by group VI and minimum in group III. In present study the increased level of lipid peroxidation in erythrocytes of affected horses are indication of elevated oxidative stress (Chaudhuri *et al.*, 2008). Thus, higher production of peroxy radicals and consequent elevated LPO concentration renders the erythrocytes more fragile and prone to lysis. Therefore, in term of improvement in LPO, SOD and catalase concentration in treated groups of equines best recovery was assessed in Group II followed by group VI and minimum in group III.

#### **Therapeutic Efficacy on the Basis of Percent Recovery Assessment**

Recovery assessment was done on the basis clinical improvement in terms of disappearance of clinical signs & parasitological examination, the percent recovery shown by the diminazine aceturate (group I) and diminazine aceturate with vitamin- E (group II) on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment was found to be 100 percent. The percent recovery shown by isometamidium chloride hydrochloride (group V) and isometamidium chloride hydrochloride with vitamin- E (group VI) on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment was found to be 83.33 percent and 100 percent respectively. The percent recovery shown by quinapyramine sulphate (group III) and quinapyramine sulphate with vitamin- E (group IV) on day 7<sup>th</sup> and day 14<sup>th</sup> post-treatment was found to be 50 percent and <100 percent respectively.

**Table 3:** Oxidative stress parameters in various groups of equines suffering from *Trypanosoma evansi* infection (Mean  $\pm$  SE)

| Groups | LPO (nmolMDA/mg Hb)            |                                |                                | SOD (U/mgHb)                  |                               |                                | Catalase (U/mgHb)              |                               |                               |
|--------|--------------------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|
|        | 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>          |
| I      | 21.93 $\pm$ 0.60 <sup>Bb</sup> | 16.7 $\pm$ 0.12 <sup>Ca</sup>  | 16.04 $\pm$ 0.04 <sup>Ca</sup> | 3.15 $\pm$ 0.06 <sup>Aa</sup> | 4.18 $\pm$ 0.04 <sup>Bb</sup> | 4.53 $\pm$ 0.07 <sup>Dc</sup>  | 1.74 $\pm$ 0.16 <sup>Ba</sup>  | 2.58 $\pm$ 0.09 <sup>Db</sup> | 2.91 $\pm$ 0.10 <sup>Db</sup> |
| II     | 21.98 $\pm$ 0.45 <sup>Bc</sup> | 14.8 $\pm$ 0.09 <sup>Bb</sup>  | 13.99 $\pm$ 0.04 <sup>Ba</sup> | 3.05 $\pm$ 0.19 <sup>Aa</sup> | 5.7 $\pm$ 0.18 <sup>Cb</sup>  | 6.23 $\pm$ 0.10 <sup>Ec</sup>  | 1.83 $\pm$ 0.14 <sup>Ba</sup>  | 3.28 $\pm$ 0.05 <sup>Eb</sup> | 3.9 $\pm$ 0.06 <sup>Ec</sup>  |
| III    | 21.9 $\pm$ 0.32 <sup>Bc</sup>  | 18.98 $\pm$ 0.09 <sup>Fb</sup> | 18.21 $\pm$ 0.14 <sup>Fa</sup> | 3.16 $\pm$ 0.05 <sup>Aa</sup> | 3.56 $\pm$ 0.06 <sup>Ab</sup> | 3.92 $\pm$ 0.08 <sup>Ac</sup>  | 1.41 $\pm$ 0.06 <sup>Aa</sup>  | 1.83 $\pm$ 0.05 <sup>Ab</sup> | 1.98 $\pm$ 0.04 <sup>Ac</sup> |
| IV     | 21.77 $\pm$ 0.41 <sup>Bc</sup> | 17.82 $\pm$ 0.08 <sup>Db</sup> | 16.92 $\pm$ 0.16 <sup>Da</sup> | 3.13 $\pm$ 0.04 <sup>Aa</sup> | 3.99 $\pm$ 0.03 <sup>Bb</sup> | 4.27 $\pm$ 0.06 <sup>Bc</sup>  | 1.34 $\pm$ 0.06 <sup>Aa</sup>  | 2.16 $\pm$ 0.04 <sup>Bb</sup> | 2.49 $\pm$ 0.04 <sup>Cc</sup> |
| V      | 21.7 $\pm$ 0.47 <sup>Bb</sup>  | 18.39 $\pm$ 0.18 <sup>Ea</sup> | 17.66 $\pm$ 0.11 <sup>Ea</sup> | 3.12 $\pm$ 0.06 <sup>Aa</sup> | 3.75 $\pm$ 0.06 <sup>Ab</sup> | 4.11 $\pm$ 0.09 <sup>ABc</sup> | 1.57 $\pm$ 0.09 <sup>ABa</sup> | 2.01 $\pm$ 0.06 <sup>Bb</sup> | 2.27 $\pm$ 0.07 <sup>Bc</sup> |
| VI     | 22.06 $\pm$ 0.46 <sup>Bb</sup> | 16.74 $\pm$ 0.13 <sup>Ca</sup> | 16.05 $\pm$ 0.03 <sup>Ca</sup> | 3.12 $\pm$ 0.06 <sup>Aa</sup> | 4.09 $\pm$ 0.04 <sup>Bb</sup> | 4.48 $\pm$ 0.09 <sup>CDc</sup> | 1.59 $\pm$ 0.11 <sup>ABa</sup> | 2.34 $\pm$ 0.08 <sup>Cb</sup> | 2.63 $\pm$ 0.04 <sup>Cc</sup> |
| VII    | 13.07 $\pm$ 0.07 <sup>A</sup>  | 12.81 $\pm$ 0.16 <sup>A</sup>  | 12.93 $\pm$ 0.13 <sup>A</sup>  | 7.12 $\pm$ 0.04 <sup>B</sup>  | 7.14 $\pm$ 0.06 <sup>D</sup>  | 7.16 $\pm$ 0.03 <sup>F</sup>   | 5.14 $\pm$ 0.03 <sup>C</sup>   | 5.22 $\pm$ 0.02 <sup>F</sup>  | 5.2 $\pm$ 0.01 <sup>F</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

Therefore, on the basis of percent recovery assessment diaminazine aceturate alone or in combination with Vitamin E was found to best, followed by isometamedium along with vitamin E and quinopyramine sulphate alone or in combination with the Vitamin E, found to be least effective in treating clinical trypanosomiasis in equines. During entire study no any adverse reactions were observed after administration of antitrypanosomal drugs, this might be due to fact we had instituted proper supportive therapies before administration of antitrypanosomal drugs. In present investigation single dose administration of isometamedium and quinopyramine 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 V & VI and two animals of group III & IV 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 isometamidium chloride hydrochloride and quinopyramine sulphate could leads to development of resistance among trypanosomes (Howes *et al.*, 2011).

### Conclusion

In conclusion it can be said from present study that diaminazine aceturate along with vitamin E and other supportive therapies in the recommended doses could be used to treat clinical cases of *Trypanosoma evansi* infection in equines with great success than other antitrypanosomal drugs currently available.

### Acknowledgement

Authors are thankful to the Dr S. K. Garg, Dean, COVSc &AH and Dr P K Shukla, Dean Post graduate studies DUVASU, Mathura, for providing necessary facilities and support during the investigation.

## References

1. Abeer A, Abd EB and Shaymaa IS. (2011). Clinicopathological and Cytological Studies on Naturally Infected Camels and Experimentally Infected Rats with *Trypanosoma evansi*. *World Applied Sciences Journal*, 14: 42-50.
2. Anosa VO. (1988a). Haematological; and biochemical changes in human and animal trypanosomosis. Part II: *Revue d' Elevage et de Medecine Veterinaire des Pays Tropicaux* 4:65-78.
3. Chaudhuri S, Varshney JP and Patra RC. (2008). Erythrocytic antioxidant defense, lipid peroxides level and blood iron, zinc and copper concentrations in dogs naturally infected with *Babesia gibsoni*. *Res. Vet. Sci.* 85: 120–124.
4. Hilali M, Abdel GA, Nassar A and Abdel WA. (2006). Hematological and biochemical changes in water buffalo calves (*B. bubalis*) infected with *T. evansi*. *Vet. Parasitol.* 139: 237- 43.
5. Howes F, Da-Silva AS, Athayde CL, Costa MM. and Corrêa MMB. (2011). A new therapeutic protocol for dogs infected with *T. evansi*. *Acta Scientiae Veterinariae.* 39(3): 988.
6. Kumar H, Gupta MP, Sidhu P K, Mahajan V, Bal MS, Kaur K, Verma AS and Singla LD. (2012). An outbreak of acute *T. evansi* infection in crossbred cattle in Punjab. *India Journal of Applied Animal Research.* 40: 256-259.
7. Kumar RM, Kamili NM, Saxena A, Dan A, Dey S. and Raut SS. (2011). Disturbance of oxidant/antioxidant equilibrium in horses naturally infected with *T. evansi*. *Vet. Parasitol.* 180: 349–353.
8. Laha R. and Sasmal NK. (2008). Endemic status of *Trypanosoma evansi* infection in a horse stable of eastern region of India – a field investigation. *Trop. Anim. Health Prod.* 40: 357–361.
9. Silva RAMS, Victório AM and Ramirez L. (1997). Effects of *Trypanosoma evansi* on the blood chemistry and hematology of coatis (*Nasua nasua*) naturally infected in the Pantanal, Brazil. *Mem. Inst. Oswaldo Cruz.* 92:110.
10. Thrushfeild M. (2008). *Veterinary Epidemiology.* 3<sup>rd</sup> ed. Blackwell Publishing Ltd, UK.
11. WHO. (1998). Control and surveillance of African trypanosomiasis. World Health Organization, Geneva, Technical Report Series No.881.