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Clinico-biochemical and chemotherapeutic evaluation in bubaline trypanosomosis

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Abstract
The present study was undertaken on buffaloes of the Mumbai region suffering from pyrexia, inappetence and dullness. The blood smears of these buffaloes stained with Fields stain were examined to confirm the presence of *Trypanosoma evansi*. The affected animals were divided into two groups (Group I and Group II), each group consisting of 11 buffaloes. Group I animals were treated with a single dose of diminazene aceturate @ 7.4 mg/kg b. wt. and group II with quinapyramine sulphate & chloride @ 7mg/kg b. wt. along with a common supportive therapy including fluids, B. complex and liver tonics, respectively. The serum biochemical profile of infected buffaloes revealed that the levels of blood GLU, TP and ALB significantly declined and levels of AST, ALT, ALP, BUN and TBIL were markedly increased. The altered values were restored to the normal range on day 15 post-treatment in both groups. The efficacy of quinapyramine sulphate & chloride, and diminazene aceturate was found to be 100 and 89.72 per cent in group II and group I animals, respectively. Group II animals treated with quinapyramine sulphate & chloride showed faster clinical recovery with early clearance of parasitaemia and marked improvement in biochemical indices than group I animals with diminazene aceturate.

Keywords: Buffaloes, biochemical profile, chemotherapeutic evaluation, *Trypanosoma evansi*

Introduction
Trypanosoma evansi infection is widely distributed across the globe, affecting a wide range of domestic and wild animals. The disease caused by *Trypanosoma evansi* is unpredictable and often goes unnoticed in large ruminants, particularly in buffaloes as they commonly suffer from chronic to subclinical form leading to loss of production and occasionally acute form of trypanosomosis causing high mortality.
Due to subclinical infection, the incidence of trypanosomosis in cattle and buffaloes goes unnoticed in India and buffaloes may act as reservoirs (Jaiswal *et al*., 2015) [18]. Cattle and buffaloes are considered to be the main source of infection to other susceptible animals. Although the diagnosis of haemoprotozoan diseases based on biochemical changes is not routinely practised in field conditions. But in present days biochemical analysis of complicated cases is being increasingly employed compared to the past. It is need for the day to assess the biochemical profiles of the infected animals. The biochemical alterations thus can offer an important clue for the diagnosis of the disease in the field conditions. Moreover, it can serve as an important biomarker in disease diagnosis, particularly in the absence of affordable, reliable and user-friendly diagnostic protocols. Keeping this fact in view, the present study was undertaken to evaluate the efficacy of two therapeutic regimens based on parasite clearance, overall improvement in biochemical values and clinical recovery after the treatment.

Materials and Methods
Blood smears of suspected buffaloes suffering from anorexia, dullness and pyrexia were examined for confirmation of *Trypanosoma evansi* by stained blood smear examination. Twenty-two buffaloes positive for trypanosomosis with varying degrees of parasitaemia were subjected to detailed clinical examination and were equally divided into two treatment groups (group I and group II).

Animals of group I were treated with diminazene aceturate @ 7 mg/kg b. wt. i/m and group II with quinapyramine sulphate & chloride @ 7.4 mg/kg body weight s/c in group II animals as a single dose along with meloxicam, dextrose saline, irons dextran, haematinic mixtures, B. complex and liver tonics at recommended doses for three days which included Inj. B. complex, dextrose and liver tonic respectively. About five ml blood was collected from the jugular vein of infected animals for routine serum biochemistry pre and post treatment. And two ml of blood was collected into fluoride vials for the estimation of blood glucose using a prietest™ in-nitrogen kit (Robonik India Pvt. Ltd.). The serum samples were used for the estimation of total proteins, albumin, globulin, bilirubin, BUN, creatinine, serum ALP, AST and ALT using priestest™ in vitro diagnostic test kit (Robonik India Pvt. Ltd.). The biochemical analysis of serum samples was done using fully automated random access biochemical analyser (Falcom 260, ARK Diagnosis) as per the instructions of the manufacturer.

Blood smears of the infected buffaloes were examined microscopically to check parasitaemia on day 0 (pre-treatment), day 1 to 7 and day 15 post-treatment. The efficacy of both trypanocidal drugs was determined based on clearance of parasitaemia, and improvement in biochemical values post-treatment. Further, the results were subjected to statistical analysis using paired t-test (WASP 2.0, HTTP: //www.ccari.res.in/wasp2.0/index.php.) and Snedecor and Cochran (1994) [38].

Results and Discussion

Biochemical findings

The effect of treatment on biochemical parameters in Group I and Group II animals was compared and it was found that changes in biochemical values of animals of both groups after treatment were variable. These biochemical values were found to improve in both treatment groups which were treated with diminazene aceturate and quinapyramine sulphate and chloride respectively (Tables 1 and 2). However, the improvement in the values of Group II animals was comparatively at par with higher margins than those of Group I. The AST, ALT and ALP mean values were significantly higher in both treatment groups. The present findings validate the findings of previous workers *viz*. Hussain *et al.* 2016 [17]; Takeet *et al.* 2009 [37]; Bal *et al.* (2014) [5]; Mishra *et al.* 2017 [22] and Pandya *et al.* 2018 [24]. In the present study higher values of AST and ALT in infected animals could be due to hepatotoxicity causing permeability and leakage of these lysosomal enzymes increasing the release of enzymes (Choudhary *et al.,* 2003) [13]. Moreover, Kaneko *et al.* (2008) [20] reported that there is erythrophagocytosis caused by parasites after peak parasitaemia due to these enzymes. The mean values of BUN in infected animals were found to be high significantly. The trend was noted by previous workers (Hilali *et al*., 2006; Bal *et al.,* 2014) [16, 5].

The BUN values significantly increased due to malfunction of the kidney by the endotoxins released by the trypanosomes and also increase in BUN could be due to the catabolic breakdown of proteins as a result of fever (Kadima *et al.,* 2000 and Coles 1986) [19, 12]. In the present study mean value of BUN was improved after treatment to the normal range. These findings corroborate with the findings of Bal *et al.* (2014) [5], Aulakh (2005) [4] and Rakesh Kumar (2015) [30] who reported significant improvement in BUN within seven days to one-month duration after treatment by antitrypanosomal drugs while Birajdar (2007) [9] reported non-significant variation in BUN value before and after treatment. In the present study, significantly high mean levels of creatinine were noticed before treatment in affected animals. Our finding corroborates with the findings of Bal *et al.* (2014) [5], Rakesh Kumar (2015) [30] and Birajdar (2007) [9]. The increased creatinine level in infected buffaloes might be due to kidney dysfunction. However, it is not a consistent finding as there can be renal injury associated with glomerular dysfunction due to the deposition of antigen and antibody complexes in glomeruli or destruction of erythrocytes at a large scale resulting in kidney dysfunction (Damyanti *et al*., 1994 and Anosa 1988) [14, 3].

A gradual increase in total protein levels during the post-treatment study period indirectly indicates the clearing of the organisms from the blood circulation and cessation of immune stimulation. These findings are in agreement with observations of Pandya *et al.* (2018) [24], Mishra *et al.* (2017) [22], Kaneko *et al.* (2008) [20], Shrinivasulu (2011) [35] and Puri (2009) [27], reduced level of serum protein in buffaloes infected with *T. evansi* is a result of hepatic insufficiency and gluconeogenesis from protein resources (Ramkrishna and Yoganand 2008) [31]. These findings of present investigations are similar to the findings of Hilali *et al.* (2006) [16] and Aulakh *et al.* (2005) [4] and Abenga and Anosa (2005) [1] reported low albumin and high levels of globulin in vervet monkeys. The significant elevation in globulin might be due to the presence of trypanosomes which activate the host immune system thereby resulting in antibody production to combat *T. evansi* infection in affected animals and an increase in albumin level might be due to impairment of the liver by degenerative changes in *T. evansi* infection (Aulakh *et al.,* 2005) [4]. A decrease in serum albumin can be related to liver injury as albumin is produced only in this organ (Kaneko *et al.,* 2008) [20]. However, Pincus *et al*. (1999) [25] confirmed that the total protein and albumin can be found at normal levels even in the damaged liver unless hepatitis involves a major part of it. In the present study, the albumin and globulin values in both groups' animals started showing improvement towards the normal range on the 15th day after treatment. A significant elevation in TBIL was noticed in *T. evansi* infected animals of both the groups (I and II) on day 0 before treatment, which may be attributed to erythrocytic membrane injury due to the lashing action of trypanosomes and release of haemolysin as a result of enhanced erythrocytic destruction. These observed changes are consistent with reports from studies on buffalo, cattle and other animals by Kadima *et al.* (2000) [19], Hilali *et al.* (2006) [16], Sahu (2008), Ramakrishna and Yoganand (2008), Birajdar (2007) [9], Takeet *et al.,* (2009) [37] and Shrinivasulu (2011) [35].

In terms of improvement in TBIL levels in treated animals, the best results were obtained in Group II as compared to Group I buffaloes. Similarly, Aulakh *et al.* (2005) [4], Birajdar (2007) [9] and Bal *et al.* (2014) [5] also observed improvement in TBIL levels within seven days to one month post-treatment. In the present investigations, the blood glucose level in buffaloes infected with *T. evansi* infection of both groups was low significantly ($p<0.01$). Hypoglycemia was also reported in buffaloes and cattle by Hilali *et al.*, (2006) [16], Birajdar (2007) [9], Puri (2009) [27],

Shrinivasulu (2011) [35] and Mishra *et al*., (2017) [22]. In trypanosomosis, marked pathogenesis is attributed to rapidly developing hypoglycemia due to liver dysfunction or more utilisation of blood glucose by multiplying trypanosomes and malfunctions of organs like the pancreas and liver which take an active part in gluconeogenesis (Soulsby, 1982) [34]. Similarly, Puri (2009) [27], Shrinivasulu (2011) [35] and Bal *et al.* (2014) [5] also reported improvement in glucose levels seven days to one-month duration after treatment.

Parasitaemia and regression of clinical signs post-treatment

In group, I, the average level of parasitaemia was 12.94 tryps /high power field (HPF) before treatment which reduced to an average of 4.83 tryps/HPF and 1.33 tryps /HPF on day 1 and day 2 respectively. However, the parasitaemia was completely cleared in all animals on day 3 post-treatment. 6 animals had average parasitaemia < 4.6 tryps/HPF and were completely cleared only in one animal on day 1 while the parasitaemia was cleared from seven animals on day 2 post-treatment and in the rest of 3 animals was cleared on day 3 after the treatment (Table 3). The animals were active, and alert and showed improvement in appetite after 3 days post-treatment and overall recovery was observed on the 15th day after treatment.

In group II, the average parasitaemia was 12.54 tryps / HPF before treatment (day 0) with three animals having high parasitaemia of 28.6 to 30 tryps / HPF. This parasitaemia was reduced to an average of 3.94 tryps/HPF on day 1 post-treatment. The parasitaemia was completely cleared from the remaining 11 animals in the group on day 2 post-treatment. On day 1 post-treatment, only three animals had average parasitaemia of 10.4, 10.8 and 11.2/HPF respectively while it was completely cleared from the blood of 8 animals of group II. On day 2 post-treatment, parasitaemia was cleared completely from the remaining animals indicating 100 per cent elimination of trypanosomes in group II animals (Table 3). However, clearance of parasitaemia was only 89.72 per cent in group I which took a day more to clear the parasitaemia to 100 per cent as compared to group II animals as clearance of parasitaemia and regression in clinical symptoms were noticed on day 2nd and overall recovery was observed on day 15thpost-treatment.

Effect of chemotherapy on biochemical parameters

Biochemical values were improved on day 15 in animals of both groups after the treatment. However, substantial improvement in the mean values of Group II animals treated with quinapyramine sulphate and chloride was comparatively at par with higher margins than those of Group I which received therapy of diminazene aceturate. However, animals of Group I showed the values of ALT, AST, ALB, GLB and CREAT were still on the higher side in 3, 2, 1, 3 and 3 buffaloes. The findings of the present study corroborate with the findings of Birajdar (2007) [9] who reported the absence of trypanosomes in buffaloes treated with berenil (7mg/kg b. wt. i/m) and antrycide prosalt (7.4 mg/kg b. wt. s/c), showed considerable clinical recovery and improvement in the blood parameters, except the slight degree of anaemia in few animals 10 days post-treatment. The utility of diminazene aceturate in *T. evansi* infection in bovines was also studied by Singh and Joshi (1991) [33] who reported 100% efficacy based on clinical recovery and clearance of parasites from the blood smears. In contrast, Soulsby (1982) [34] and Bhatia *et al*. (2006) and Shrinivasullu (2011) [35] reported inadequate efficacy of diminazene against *T. evansi* irrespective of species of hosts. Bal *et al.* (2014) [5] reported that treatment of infected animals with Triquin (quinapyramine sulphate and chloride) @ 5mg/kg b. wt. showed improvement in general body condition within two weeks and the results of PCR were also negative for *T. evansi* in all the blood samples. Further, they reported the highest efficacy in terms of restoration of biochemical values after one-month post-treatment. Similarly, Shrinivasullu (2011) [35] and Suman Kumar *et. al.* (2009) [36] also reported clinical response on the 3rd day and complete recovery by the 10th day of post-treatment using quinapyramine sulphate and chloride @ 4.4 mg/kg. b. wt. s/c. along with the same supportive therapy. Various researchers *viz* Rajesh *et al.* (2010) [28], Rajguru *et al.*, (2000) [29], Ajayi *et al*. (2013) [2], Omoja *et al.* (2012) [23] and Bal *et al.* (2014) [5], Kumar *et al*. (2015) [30], and Ponnudurai *et al.* (2015) reported 100 per cent efficacy in different species of animals suffering from trypanosomosis after treatment with either of the trypanocidal drugs. Bidyasankar *et al.* (2005) [8] compared the efficacy of Triquin and Berenil in *T. evansi*-infected buffaloes and found that the overall recovery rate was earlier in Triquin-treated animals compared to Berenil-treated animals.

On the contrary, Ramkrishna and Yoganand (2008) [31] reported that there was no significant difference in the recovery rate among animals treated with Triquin and Berenil, though Triquin had the highest efficacy compared to Berenil which was similar to the observations of the present study. The observations of the present study support the findings of Dixit *et al*. (2007) [15] who also observed that a combination of quinapyramine sulphate and quinapyramine chloride used as antrycide prosalt @ 7.4 mg/kg body weight showed excellent efficacy against *T. evansi* infection in cattle and buffaloes. Brander (1982) [10] also reported less efficacy of diminazene aceturate against not only *T. evansi* but also against the species belonging to the brucei group. Contrary to present study results, Ramkrishna and Yoganand (2008) [31] opined that diminazene aceturate is the drug of choice for treating trypanosomosis with high potency, safety margin and fewer adverse effects in buffaloes.

Table 1: Biochemical profile of *T. evansi* infected buffaloes (Group I) pre-and post-treatment with diminazene aceturate.

Sr. No	Parameter	Mean ± SE Before treatment	Mean ± SE After treatment	*Normal Range
1.	ALT (IU/L)	65.36±1.04**	58.09±0.99	52-60
2.	AST (IU/L)	175.55±0.89**	161.73±2.43	155-173
3.	ALP (IU/L)	143.82±2.41**	133.27±2.58	30-145
4.	TP (gm/dl)	7.34±0.10**	8.09±0.02	7.99-8.99
5.	ALB (gm/dl)	2.95±0.01**	3.61±0.008	3.60-3.63
6.	GLB (gm/dl)	5.71±0.90**	5.42±0.08	4.25-5.47
7	TBIL (mg/dl)	0.73±0.33**	0.39±0.01	0.3-0.4
8.	BUN (mg/dl)	27.94±0.48**	24.26±0.24	23.83-26.63
9.	CREAT (mg/dl)	2.82±0.51**	2.58±0.01	2.5-2.62
10.	GLU (gm/dl)	41.71±0.95**	54.06±0.83	50-60

NS–Non-Significant (P>0.05), *Significant (P<0.05), **Highly Significant (P<0.01)

*****(Bhikane and Kawitkar, 2000)

Table 2: Biochemical profile of *T. evansi* infected buffaloes (Group II) pre and post-treatment with quinapyramine sulphate and chloride.

Sr. No	Parameter	Mean ± SE Before treatment	Mean ± SE After treatment	*Normal Range
1.	ALT (IU/L)	82.36±3.18**	58.73±1.02	52-60
2.	AST (IU/L)	177.36±2.07**	162.27±2.08	155-173
3.	ALP (IU/L)	144.64 ±1.20**	138.36±0.91	30-145
4.	TP (gm/dl)	6.07±0.11**	8.36±0.11	7.99-8.99
5.	ALB (gm/dl)	2.97±0.07**	3.62±0.003	3.60-3.63
6.	GLB (gm/dl)	5.89±0.01**	5.45±0.12	4.25-5.47
7	TBIL (mg/dl)	0.64±0.06**	0.39±0.01	0.3-0.4
8.	BUN (mg/dl)	29.24±0.84**	25.97±0.21	23.83-26.63
9.	CREAT (mg/dl)	2.78±0.04**	2.60±2.36	2.5-2.62
10.	GLU (gm/dl)	42.02±0.96**	54.63±0.80	50-60

NS–Non-Significant (P>0.05) * Significant (P<0.05) * * Highly Significant (P<0.01)
*****(Bhikane and Kawitkar, 2000)

Table 3: Post-treatment parasitaemia and regression of clinical signs.

Day	Group I Diminazene aceturate	% Efficacy	Group II Quinapyramine sulphate and chloride	% Efficacy
	Avg. Parasitaemia		Avg. Parasitaemia	
1	12.95		12.55	
2	4.83	62.70	2.95	76.49
3	1.33	89.72	0	100
4	0	100	0	
5	0		0	
6	0		0	
7	0		0	
15	0		0	

Conclusion

The combined treatment of quinapyramine sulphate and chloride along with supportive therapy showed more encouraging results in group II animals than in group I treated with diminazene aceturate in terms of early clearance of parasitaemia, marked improvement in biochemical values and fast clinical recovery from infection.

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