

SHORT COMMUNICATION

Comparative Efficacy of Medicinal Plant Formulation, Isometamidium Hydrochloride, and Diminazene Aceturate Against Natural *Trypanosoma evansi* Infection in Cattle

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ABSTRACT

This study was carried out to determine the risk factors and evaluate the therapeutic efficacy of selected Medicinal Plants Formulations (MPF) of *Nicotiana tabacum*, *Azadirachta indica*, *Calotropis procera*, and *Trachyspermum ammi* in comparison to isometamidium hydrochloride (IMH) and diminazene aceturate (DMA) against naturally infected cattle with *Trypanosoma evansi*. Briefly, blood samples were collected from 500 crossbred cattle followed by examination through direct smear and thick smear. A total of 120 parasite positive cattle were selected and divided into four groups (30 each) treated with IMH 0.5mg/kg IM (Group A), DMA 5.0mg/kg IM (Group B), MPF 200mg/kg OP (Group C), and non-infected non-treated (Group D). Prevalence (%) in 1) malnourished cattle, 2) aged 3+ years in, 3) open housing systems and 4) near dung pile locations was 38, 17, 23 and 19%, respectively, demonstrating higher infection rates. IMH and DMA showed 100% recovery, with significant symptom relief but notable side effects. MPF, though slower in action, achieved 90% recovery with no observed side effects. MPF found a promising, side-effect-free alternative treatment for trypanosomiasis and supports the phytotherapy in veterinary.

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INTRODUCTION

Trypanosoma (T.) evansi is globally prevalent parasite causing significant direct and indirect economic losses from 4-4.5 billion US dollars, annually (Seangseerattanakulchai et al., 2021). The invertebrate host of *T. evansi* is tabanid flies, commonly called horse flies (i.e., *Tabanus striatus* and *Tabanus reducens*). The versatility of the vector flies has allowed them to expand across a wider geographic area (Nguyen et al., 2024). In their vertebrate host, it may infiltrate the joints and central nervous system and be found in the blood and lymphatic system (Metwally et al., 2021). The acute type of infection shows high fever alongside severe anemia, which is often deadly if parasitemia is present (Ocampos et al., 2024). The subacute type, which is more gradual but can also be fatal, is signified by intermittent fever, swelling and corneal opacity (Gupta et al., 2023).

Chemical drugs are usually the primary line of treatment against parasitic infections. Quinapyramine sulphate (Prayag et al., 2023), suramin (Abdullahi et al., 2023), diminazene

aceturate (DMA) and isometamidium hydrochloride (IMH) are commonly used drugs against *T. evansi* in cattle (Bello et al., 2023). However, the resistance of *T. evansi* against these drugs has already been reported (Mdachi et al., 2023). On the other hand, traditional drugs such as IMH and DMA are toxic to *T. evansi* by inhibiting DNA and energy metabolism but tend to induce toxicity and resistance. In the backdrop of above, there is a dire need for alternative treatment options (Soliman et al., 2024). The plant compounds from MPF exhibit action through membrane disruption and immune activation, which is safer and more sustainable. Thus, the present study aimed to find the associated risk factors and to evaluate the combined efficacy of selected MPF in comparison with IMH and DMA against naturally infected cattle with *T. evansi*.

MATERIALS AND METHODS

Study area: The present research was conducted in the Muzaffargarh District (30.1392° N, 71.0973° E) in Southern Punjab, Pakistan (Fig. 1).

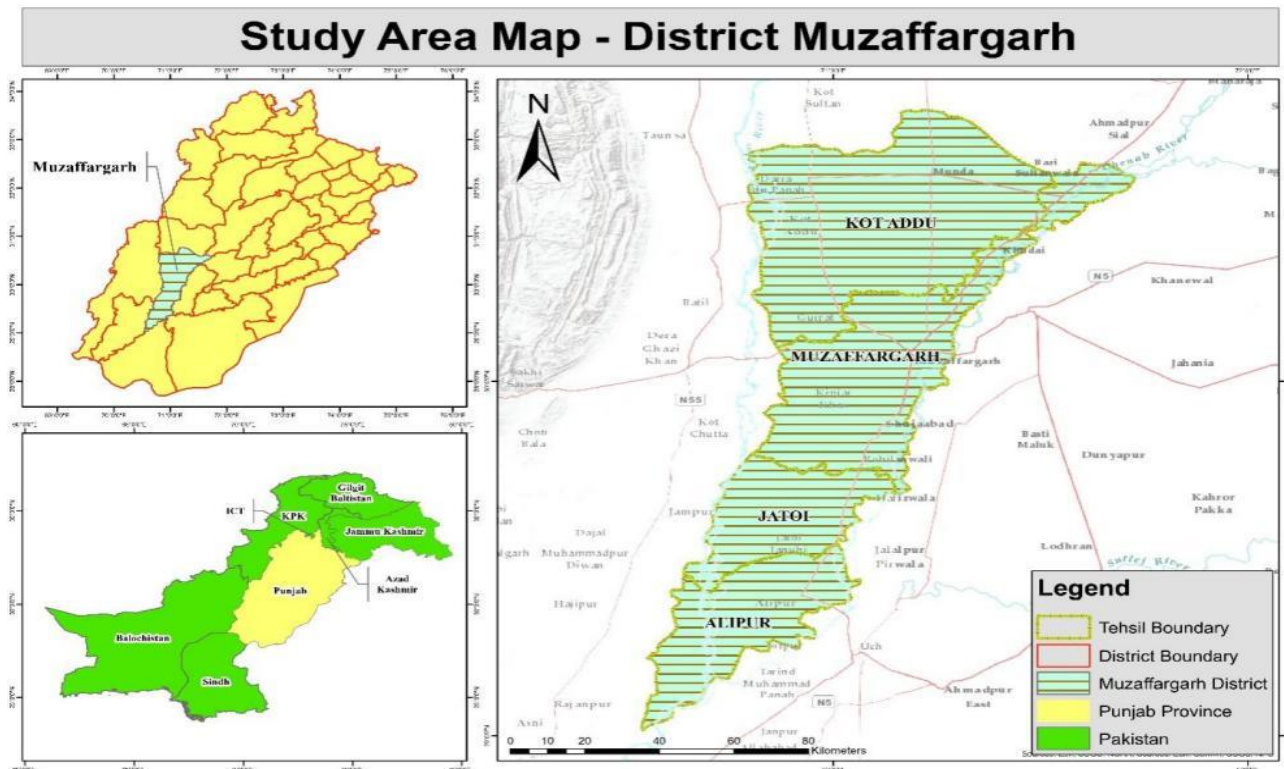


Fig. 1: Geographical distribution of sampling sites (Muzaffargarh, Kot Addu, Ali Pur and Jatoi).

Sample collection, processing and risk factor assessment: Blood samples were collected in EDTA vacutainers tubes directly from the jugular vein of 500 crossbred cattle from various location of study area. Clinical signs and symptoms of all animals were also observed (Fig. 2A). The collected samples were then examined through direct smear and thick smear blood films as per standard protocols. Giemsa staining (10% giemsa solution for 30 minutes at room temperature) was performed by following Benjamin's instructions. The presence of *T. evansi* was confirmed by the microscopic examination of blood film (Fig. 2B). Differential identification of *T. evansi* was based on the morphology described by Desquesnes et al. (2013) and Sazmand et al. (2022). It exhibits shorter free flagellum and the terminal kinetoplast. The data about various risk factor was also recorded on pre-designed questionnaire. A total of 120 crossbred cattle which were found positive for *T. evansi* were selected for further trial.

Experimental design: The selected plants were combinedly used to make aqueous-methanolic extract as per standard procedures. Cattle were divided into the following four groups: Group A: treated with IMH; Group B treated with DMA; Group C treated with MPF while Group D was kept as non-infected non-treated. Each group contains 10 animals (n=10) with triplicates. IMH (0.5mg/kg) and DMA (5.0mg/kg) per animal was administered intramuscularly (IM) in two doses: one on the first day and the second 48 hours after the first dose. MPF (200mg/kg) was administered orally (OP) in three doses after 48 hours of every dose. The study was undertaken in compliance with the institutional guidelines for the care and use of experimental animals issued by ethical committee, College of Veterinary and Animal Sciences, Jhang.

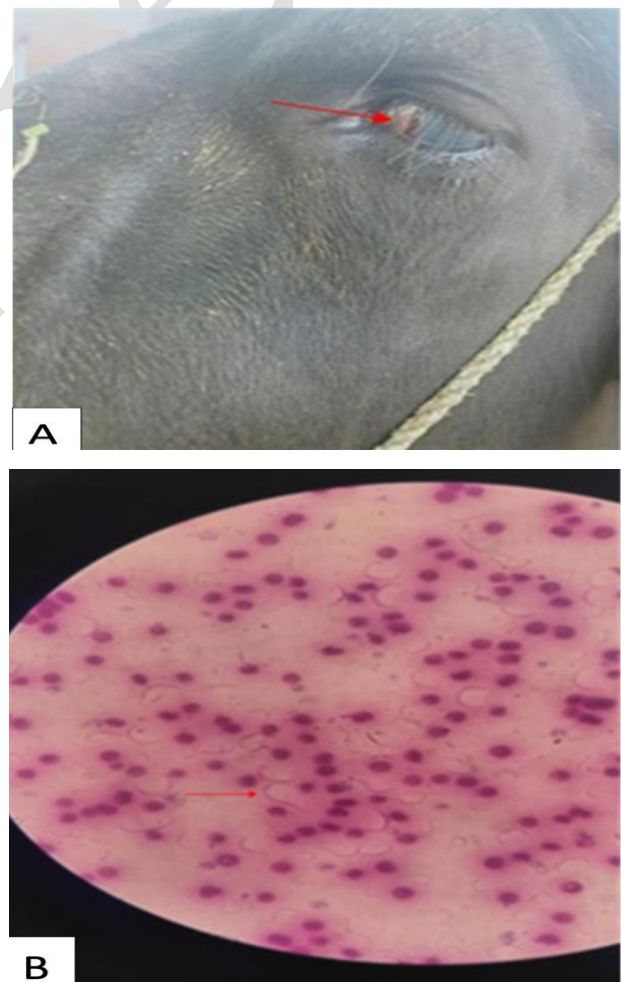


Fig. 2: Clinical picture diseased animals A: Cattle with corneal opacity (a significant clinical sign of disease), B: Photomicrograph of *T. evansi* in blood samples of cattle.

Post treatment comparative efficacy: Blood samples were systematically collected from each treated cattle every 12 hours post-first dose and subjected to buffy coat and packed cell volume (PCV) determination. The clinical signs and symptoms were evaluated throughout five days, and changes (if any) were noted. Therapeutic efficacy of MPF on the cattle calculated by:

$$\text{Therapeutic Efficacy \%} = \frac{\text{No. of recovered animals}}{\text{Total number of animals}} \times 100$$

Statistical Analyses: Statistical analysis was done using IBM SPSS²¹. Chi-square tests and one-way ANOVA were used to assess associations and treatment effects, with significance set at $P < 0.05$.

RESULTS

The prevalence of various risk factors in 1=malnourished 2=cattle aged 3+ years, 3) in 3=open housing systems and 4=near dung pile locations was found to be 38, 17, 23 and 19%, respectively, demonstrating higher infection rates ($P < 0.05$) as given in Table 1. IMH and DMA showed 100% recovery after three to four days, with significant clinical sign relief but with notable side effects (increased salivation, diarrhea and ataxia). MPF, though slower in action, achieved 90% recovery with no observed side effects within four to five days (Fig. 3; Table 2). The absence of *T. evansi* in the buffy coat and a sustained increase in PCV were observed in all treatments.

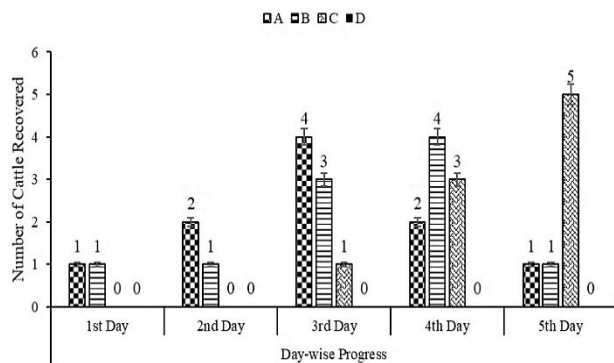


Fig. 3: Day-wise findings exhibited a progressive decline in clinical symptoms and parasitemia across all treatment groups for five consecutive days. Groups A (IMH), B (DMA) and C (MPF) showed significant improvement, with decreasing numbers of *T. evansi* on blood smears, while Group D (non-infected/non-treated) was clinically normal. The values shown in each column express the state of recovery of cattle on each respective day.

Table 1: Prevalence of Trypanosomiasis (*T. evansi*) and associated risk factors in cattle.

Risk Factors	Sub Parameter	Total cattle (n)	Positive cattle (n)	Negative cattle (n)	Prevalence (%)	P-value
Health Status	Healthy	500	55	170	11.0	0.003
	Malnourished		190	85	38.0	
Age	< 3 years	500	55	170	11.0	0.015
	> 3 years		85	190	17.0	
Housing Type	Open	500	115	99	23.0	0.002
	Tethered		106	180	21.2	
Dung Position	Pile Near to cattle	500	95	83	19.0	0.017
	Far from cattle		65	257	13.0	

Note: The same 500 cattle were analyzed across all listed risk factors. Therefore, individual animals may appear in more than one category, and totals may exceed the overall sample size.

Table 2: Day-wise comparative clinical symptoms assessment of IMH, DMA, MPF in cattle naturally infected with *T. evansi*

Days	Groups			Groups			Groups			Groups			Groups		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
	Corneal Opacity			Lymph swelling			Pulse rate			Respiration rate			Fever		
1 st Pt.	++	++	++	+++	++	+++	++	++	++	++	++	++	+++	++	+++
2 nd Pt.	+	+	++	++	++	++	+	+	++	+	++	+	++	+	++
3 rd Pt.	±	±	+	+	+	++	±	±	+	±	±	+	+	+	+
4 th Pt.	-	±	±	±	±	+	-	-	±	-	-	±	-	-	+
5 th Pt.	-	-	±	-	-	±	-	-	±	-	-	±	-	-	+

Isometamidium hydrochloride=IMH (Group A); Diminazene aceturate=DMA (Group B); Medicinal Plants Formulation (*N. tabacum*, *A. indica*, *C. procera* and *T. ammi*) =MPF (Group C). Symptom severity was scored using clinical grading for Corneal Opacity and Lymph node swelling: Severe=+++; Moderate=++, Mild=+, Very slight=±, No clinical sign= -. For Pulse rate, Respiration rate and Fever, respectively as follows: 100-120bpm=+++; 90-100bpm=++, 90-70bpm =+, 70-50bpm =±, 50-40bpm = -, 60-80bpm=+++; 50-60bpm=++, 40-50bpm =+, 30-40bpm =±, 10-30bpm = -, 104.0°F =+++; 103.0°F =++, 102.2°F =+, 101.5°F = -; Pt.=Post treatment

DISCUSSION

Trypanosomiasis is a protozoan parasitic disease, which is debilitating to the broad spectrum of domestic and wild animals. It is transmitted by hematophagous horse flies and it occurs in tropical and sub-tropical regions causing significant economic losses. Muzaffargarh and surrounding areas were used for sampling due to poor cattle grooming methods and a high rate of horseflies (Anandhabhairavi et al., 2025).

The prevalence results align with earlier studies (Okello et al., 2022) suggesting that risk factors such as Malnourished and older (>3 years) cattle are more vulnerable due to their weakened immune defense (Kanwal et al., 2025). Similarly, the higher risk in open housing and near dung piles can be attributed to increased exposure to vectors such as horse flies (Kengradomkij et al., 2025). The plants selected for this study had been empirically used by the local farmer to treat livestock diseases (Zaman et al. 2012). The synergistic phytochemical combination of *N. tabacum*, *A. indica*, *C. procera* and *T. ammi* is characterized by antiparasitic, insecticidal, anti-inflammatory and immune-enhancing activities (Nodza et al., 2022).

The fastest therapeutic response was exhibited by IMH, may be due to the trypanocidal qualities of IMH, which interfere with *T. evansi* DNA replication and oxidative metabolism (Mdachi et al., 2023). DMA also showed significant improvement slower than IMH but its frequent dosing and hepatotoxic effects need caution (Tejedor-Junco et al., 2023). MPF effectiveness were noticed from the third day onwards, and on the fifth day, there was recovery with slight clinical signs (except for one death). The complex action of phytochemicals and OP may be responsible for the comparatively late onset (Verma et al., 2023). Compounds like nicotine (Metwally et al., 2021), azadirachtin (Wylie and Merrell, 2022) and thymol (Abdullahi et al., 2023) contributed to antiparasitic, anti-inflammatory, and immunomodulatory effects. The absence of *T. evansi* and improved PCV by the end of the treatment suggests effective parasite clearance (Seangseerattanakulchai et al., 2021). MPFs achieved 90%

recovery ($P < 0.05$) with no side effects, supporting their traditional use and future optimization (Gupta et al., 2023).

It is evident that plant-based medicines are an effective alternative to synthetic trypanocides in controlling *T. evansi* infections. The plant-derived pharmaceutical class isoquinoline alkaloids, e.g., the crystalline chloride salt of berberine, had sufficient activity against *T. evansi* in vitro with an IC_{50} of 12.15 μ m and a large selectivity index against mammalian cell lines. The transcriptomic profile showed that the proteins that treat the DNA replication, redox equilibrium, and calcium signaling were significantly varied in their expression level. This shows that the mechanism of action is very complex (Verma et al., 2023). Similarly, it is revealed that a terpenoid- abundance portion of *Salvia officinalis* contained a significant decrease in parasitemia and enhancement of the hematological and biochemical parameters in *T. evansi* infected rats, which indicated its possible application in vivo (Sawerus et al., 2025). Also, the *Balanites aegyptiaca* leaf extract of infected rats contributed to the restoration of packed cell volume, levels of hemoglobin, and leukocytes, which demonstrates possible reversal of trypanosome-induced anemia and immunosuppression (Abdullahi et al., 2023). The results show the possibility of phytochemicals in breaking down major survival mechanisms of parasite and affordable means of controlling disease particularly in areas where resistance as well as cost is becoming a major challenge.

Conclusions: In the scenario of emerging resistance to anti-parasitic drugs, the MPF offered efficacy with no side effects, making it a safer, sustainable alternative. Specialized initiatives, like farmer education and vector control measures, must be taken to mitigate the economic losses caused by trypanosomiasis in Southern Punjab, Pakistan.

Authors contribution: MAZ conceptualized the study and reviewed the manuscript. TH and SA contributed to the initial drafting, while MAZ conducted the statistical analysis. TH was responsible for data collection and compilation.

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