

## Effect of Anti-Piroplasm Drug on Various Physiological Indices in Healthy Mongrel Dogs

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### ABSTRACT

In this study, we aimed to evaluate the changes in hematology, hepatic and renal profile of healthy Mongrel dogs injected with therapeutic doses of Imidocarb dipropionate (IMDP) to determine the changes in physiological indices. Six adult, male and healthy Mongrel dogs, aged above one year, weighing 15-20 kg were injected with two intramuscular doses of Imizol@ 6mg/kg body weight at 48 hours dosage interval. The hematology, renal and hepatic profile was determined on day 1 (pre-treatment) and day 2, 3, 7 (post-treatments). The activity of serum Alanine Aminotransferase (ALT) increased significantly on day 3 compared to the pre-treatment (day-1). The serum creatinine concentration was significantly elevated on day 2 ( $p < 0.05$ ) than its pre-treatment values. However, the serum Aspartate Aminotransferase (AST), Alkaline Phosphatase (AP), complete blood count (CBC), Bilirubin concentration and Blood urea nitrogen (BUN) did not differ significantly between pre-treatment and post-treatment periods. Based on the results obtained, it was concluded that IMDP may induce minor damage to hepatic and renal function, from which dogs recovered during the studied time period.

**Key words:** Antipiroplasm, Mongrel dogs, Imizol, Hematology, Babesiosis

### INTRODUCTION

Babesiosis is a tick-borne disease of economic importance and is one of the most common life threatening (Boozer and Macintire, 2003; Miyama et al., 2005) problems that affects a wide range of vertebrate hosts in tropical and subtropical regions of the world (Uilenberg, 2006). It is a well-recognized disease of veterinary importance and has shown appreciable morbidity and mortality in domestic animals including, cattle, horses, cats and dogs (Schoeman, 2009). It has also gained increasing attention as an emerging zoonotic disease problem (Vial and Gorenflot, 2006). In rural areas where dogs are commonly reared by livestock owners as watchdogs, they often have ticks on their body and are among the major risk factors for spread of babesiosis in small ruminants in Pakistan (Iqbal et al., 2011).

For the treatment of babesiosis, a range of medicines have been administered previously like Berenil and Acaprin (Zwart and Brocklesby, 1979), but Imidocarb dipropionate (IMDP) and diminazene aceturate are most commonly used (Vial and Gorenflot, 2006). In a comparative efficacy trial among these drugs, IMDP belonging to the group of diamidine derivatives (3, 3-di-2-imidazolin-2-yl-carbanilide) is an effective chemotherapeutic drug against babesiosis, both therapeutically and prophylactically (Joyner, 1981; Zintl et al., 2003; Vial and Gorenflot, 2006). Therapeutic efficacy of IMDP has been evaluated in equines in Pakistan (Rashid et al., 2009). However, less information is available regarding alteration in the hepatic and renal profile in the Mongrel dogs in Pakistan. Also there is an evidence of better survivability and resistance against

pathogens in Mongrel dogs compared to the pedigreed ones (Serge et al., 1986; McGreevy and Nicholas, 1999).

The present study was therefore, conducted to evaluate physiological and biochemical changes in healthy Mongrel dogs injected with two Intramuscular (IM) doses of IMDP at 48hour interval with particular reference to liver and kidney functions.

### MATERIALS AND METHODS

Six adult, male and healthy Mongrel dogs, aged above one year, weighing 15-20 kg were used for the trial. All the dogs used in the study were reared for experimental purpose and housed in a well ventilated and tick free isolation unit in the Pet Centre, University of Veterinary and Animal Sciences, Lahore throughout the trial. The dogs were introduced into their new environment approximately ten days before the commencement of the study for acclimatization. The dogs were kept in the cages and marked with a unique number for the purpose of identification and were fed with commercial dog food (Wagg Foods Ltd, North Yorkshire, UK) with free access to fresh water. Diets were standardized and a routine was established to eliminate the potential effects of nutrition and stress on biochemical profile. History revealed that dogs were vaccinated against rabies and de-wormed prior to the experiments. The dogs were treated with Frontline spray 100 mL (Merial Ltd, NSW, Australia) for ectoparasite control. All the dogs were subjected to a complete physical and laboratory examination on a day before the trial (day-1) to rule out any underlying disease that might affect the metabolism of the drug. As

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exclusion criteria, the animals with more than one of the laboratory values above or below normal limits were excluded from the study.

### Experimental Design

Six healthy dogs were given two intramuscular (IM) doses of Imizol (Imidocarb dipropionate; 12% w/v ICI, Pakistan Ltd.), 6mg/kg or 0.05ml/kg body weight (BW) at 48 hours dosage interval (Vial and Gorenflot, 2006). The experiment was approved by university ethical committee.

### Blood Samples Collection and Processing

Blood samples from the animals were drawn through cephalic vein on day-1 before drug administration (pre-treatment) to assess the status of their health. After injecting the first dose; the blood samples were taken on day 2, 3, and 7. The second intramuscular injection was administered on day 2 after taking the second blood sample on day 2. On each sampling, 4.0 ml of blood was drawn, in gel vacutainers without anticoagulant (for the estimation of liver and kidney function) and 1.0 ml blood in a centrifuge tube containing EDTA (for complete blood count).

### Biochemical Analysis

Liver function tests (alanine amino transferase (ALT/SGPT), aspartate amino transferase (AST/SGOT), alkaline phosphatase (ALP), bilirubin and renal function tests (blood urea nitrogen and creatinine) were performed at University Diagnostic Laboratory (UDL), UVAS, Lahore within 12 hours of the blood sample collection.

AST, ALP, ALT, Bilirubin, BUN (Linear Chemicals S.L., Barcelona, Spain) and Serum Creatinine (Spectrum Diagnostics, Hannover, Germany) were performed using commercially available kits by Semi automated Clinical analyzer (UV-VIS METROLAB, Metrolab 1600 plus, Argentina). AST, ALT, BUN investigations were performed using UV enzymatic kinetic methods and Bilirubin was performed by end point calorimetric method. ALP and serum creatinine were performed using kinetic calorimetric method.

### Hematology analyzer

Complete blood test was performed using Hematology analyzer (Abacus junior vet. Serial # 130076, Diatron GmbH wein, Austria) at UDL, UVAS, Lahore. The WBC, PLT and RBC analysis was based on the impedance method (Meo, 2004).

### Statistical Analysis

The obtained data was processed using SPSS version 13 (SPSS Inc. Chicago, US). One-way ANOVA using LSD was applied to evaluate mean differences in pre-treatment values (day-1) with post-treatment values (day 2, 3 and 7). Difference among the means of groups with  $p < 0.05$  was considered as significant.

## RESULTS AND DISCUSSION

The effect of Imidocarb dipropionate (IMDP) on hematological variables, renal profile (Mathe et al., 2007) and hepatic profile (Renata et al., 2007) has been evaluated in *Babesia* infected dogs (Irwin and Hutchinson, 1991; Angel et al., 2005; Adaszek and Winiarczyk, 2008). Effects of IMDP in the liver and kidney have also been studied previously in adult infected Mongrel dogs (Abdullah et al., 1983). In this study we aimed to evaluate the changes in hepatic and renal profile in healthy Mongrel dogs treated with therapeutic doses (6 mg/kg) of IMDP in Pakistan to determine the changes in physiological indices associated with IMDP administration in our local dogs. The overall trend in hepatic, renal and hematological profile was in agreement as reported in the previous studies, but it was observed that the deviation in the physiological indices in local Mongrel dogs was to a lesser extent compared to the pedigree breeds of dogs.

### Serum Alanine Aminotransferase (ALT/SGPT)

The activity of serum alanine aminotransferase (ALT/SGPT) increased significantly (almost 25%) on day 3 ( $p < 0.05$ ) compared to the pre-treatment (day-1) value (Table 1). One of the experimental dog showed a 3 fold increase in the activity of ALT on day 3 compared to the pre-treatment value (0.37  $\mu\text{kat/L}$ ), the serum ALT activity at this time was 1.6  $\mu\text{kat/L}$  which also exceeded the reference range of dogs (0.14-0.86  $\mu\text{kat/L}$ ). The ALT activity on day 3 was significantly different than the values on day-1 and 2. There was no significant difference between the pre-treatment value (day-1) and the values of day 2 and 7. These findings are not in agreement with Renata et al. (2007), where the serum ALT activity tended to reduce on day 5 after injecting Imidocarb (6 mg/kg) than the pre-treatment value in *B. canis* infected dogs. This discrepancy might be attributed to the fact that the dogs in these studies were already infected with babesiosis and had high ALT activity and the treatment with IMDP tended to decrease the levels of ALT activity. Kock and Kelly (1991) also reported hepatic cell necrosis due to administration of IMDP.

### Serum Aspartate Aminotransferase (AST/SGOT)

The serum aspartate aminotransferase (AST/SGOT) activity was not significantly altered at any time during the trial. However, it tended to be higher on day 3 than

pre-treatment value (day-1) (Table 1). These findings are in agreement with those of Adams et al. (1980) in calves and Ali et al. (1985) in goats who reported non-significant elevation in serum AST activity after injection of therapeutic dose (5 mg/kg) of Imidocarb. A significant elevation in serum AST activity was observed under higher dose (20 mg/kg) of IMDP in the goats (Ali et al. 1985). Meyer et al. (2005) reported a significant elevation in AST activity on day 2, 5, 8, 11 and 18 which had returned to pre-treatment levels by day 38 of the trial in healthy ponies following 4 IM doses of IMDP (4 mg/kg) in ponies at 72 hours interval. This significant elevation might be due to four repeated doses of IMDP at 72 hours interval.

### Serum Alkaline Phosphatase

The serum alkaline phosphatase activity (ALP) of day 2, 3, and 7 did not differ significantly from the pre-treatment value at any time during the trial (Table 1). A 10% increase in the values of serum alkaline phosphatase activity was seen on day 2 and 3 whereas, a 20% increase was observed on day 7 as compared to their pre-treatment values (Table 1). These findings are not in agreement with those of Ferda et al. (2013) who reported significant increase in the serum ALP activity post-treatment in infected sheep. However, the findings of Ferda et al. (2013) were 20-30 days post treatment that may suggest an effect of duration on ALP activity.

### Serum Bilirubin

The serum bilirubin concentration after the treatment did not differ significantly from the pre-treatment value at any time during the trial. However, it tended to be higher on day 3 but returned back to almost its pre-treatment value on day 7. There was a two fold increase in the mean value of bilirubin on day 3 as compared to the pre-treatment value. The hepatic profiles are summarized in Table 1.

### Serum Creatinine

The serum creatinine concentrations (mmol/L) were significantly elevated on day 2 ( $p < 0.05$ ) than its pre-treatment values. However, it returned back to almost its pre-treatment value on day 3 and day 7 (Table 2) justifying the regenerative capacity of the kidneys. Approximately 6 fold increase in the value of serum creatinine was recorded on day 2 (364.1 mmol/L). The serum creatinine concentrations of day 2 were significantly higher than day 3 and day 7. No significant difference was found between the pre-treatment values, day 3 and day 7 (Table 2). The increase in creatinine level and trend towards elevated BUN levels observed in our study suggests azotaemia which is in agreement with the previous reports by Corrier and Adams (1976), Adams and Corrier (1980), Adams (1981) and Meyer et al. (2005) in different animals. Monitoring of renal

functions before and during the treatment may be of a great value in diseased animals as marked variation in pharmacokinetics of IMDP was reported in diseased goats (Abdullah and Baggot, 1986). The effects of IMDP on biochemical profile can be more significant in hepatic and renal compromised patients. Low dose of IMDP is recommended due to its nephrotoxicity. IMDP was not reported as a safe drug by Roberson et al. (1977). It was previously suggested to use low dose of IMDP (Mathe et al., 2007), and to administer IMDP with caution in patients with possible renal involvement until further data become available on its potential nephrotoxicity in dogs. The elevated creatinine levels were reported in South African dogs having *Babesia canis* infection (Welzl et al., 2001). Similarly, Crnogaj et al. (2010) and Mathe et al. (2007) reported 133  $\mu\text{mol/L}$  and 582  $\mu\text{mol/L}$  of creatinine concentration in *Babesia* infected dogs, respectively.

### Serum Blood Urea Nitrogen

The concentration of serum blood urea nitrogen ( $\mu\text{mol/L}$ ) did not increase significantly on day 2, 3 and 7 (post-treatment) than the pre-treatment values. However, BUN concentration on day 3 was significantly higher than day 2. BUN concentration on day 7 was not significantly different than the concentration on day-1, 2, and 3 (Table 2). Similar effect was reported with therapeutic dose of IMDP in other species (Meyer et al., 2005). These findings are not in agreement with those of Corrier and Adams (1976) who reported an increase in the concentration of BUN with the increasing dose of IMDP which was attributed to the excessive re-absorption of the urea from the damaged renal tubules. The significant elevation in BUN concentration was also reported following higher doses of IMDP in healthy Nubian goats (Ali et al. 1985). However, in our study, the highest serum blood urea nitrogen concentration was 2.2 mmol/L on day 3 (post-treatment) that returned to the pre-treatment value on day 7 (1.33-1.8 mmol/L). A moderate elevation in serum BUN (10.7 mmol/L) in *Babesia* infected dogs treated with 3-6 mg/kg IMDP was also reported by Mathe et al. (2007).

### Hematological variables

No significant change in hematocrit/PCV was observed in our experiment (Table 3). These findings are not in agreement with previous studies as a significant decrease in PCV was reported in post-treatment values by Uilenberg et al. (1981), Ali et al. (1985), Meyer et al. (2005). No significant changes were observed in hemoglobin concentration and WBC count. Similar observation was previously reported in ponies administered with four IM doses of IMDP (Meyer et al., 2005). No significant alteration was observed in RBC count and PLT count in our experiment. Ali et al. (1985) also reported no significant change in hematological variables induced by normal dose of IMDP (6mg/kg) in

goats but a significant decrease in PCV, RBC count and Hb was reported by higher doses of IMDP (18-24 mg/kg). A trend towards elevated WBC count was also observed that correlates with trend towards elevation of AST activity suggesting a slight increase of WBC might be due to mild muscular necrosis at injection site (Meyer et al., 2005). However, Naz et al. (2012) reported that age, sex and season has no effect on piroplasm infection

in goats. The non-significant decrease in the values of RBC count and hemoglobin concentration observed was not in agreement with previous findings in goats by higher doses of IMDP (Ali et al., 1985). The hematological results obtained in this study indicated that the dosage regime of IMDP used had minimal effect on cellular blood components.

**Table 1** Comparison of pre and post-treatment values of hepatic profile following intramuscular administration of 2 doses of 6 mg/ kg Imidocarb dipropionate at 48 hours interval to 6 healthy dogs.

Variables	Day -1	Day 2	Day 3	Day 7
	Pre-treatment	Post-treatment	Post-treatment	Post-treatment
ALT (μkat/L)	0.37±0.1	0.46±0.0	0.81±0.2*	0.76±0.7
AST (μkat/L)	0.62±0.1	0.42±0.0	0.77±0.1	0.64±0.1
ALP (μkat/L)	1.11±0.3	0.79±0.2	1.2±0.2	1.45±0.13.1
Bilirubin (μmol/L)	9.4±4.1	7.1±1.2	19.4±7.2	8.2±2.1

\* indicates significant difference from the pre-treatment value.

**Table 2** Comparison of pre and post-treatment values of renal profile following intramuscular administration of 2 doses of 6 mg/ kg Imidocarb dipropionate at 48 hours interval to 6 healthy dogs.

Variables	Day -1	Day 2	Day 3	Day 7
	Pre-treatment	Post-treatment	Post-treatment	Post-treatment
Creatinine (mmol/L)	63.6±12.6	364.4±72*	81±10	69±18
BUN(μmol/L)	1.8±0.14	0.67±0.42	2.27±0.36	1.33±0.43

\* indicates significant difference from the pre-treatment value.

**Table 3** Comparison of pre and post-treatment values of hematological parameters following intramuscular administration of 2 doses of 6 mg/ kg Imidocarb dipropionate at 48 hours interval to 6 healthy dogs

Variables	Day -1	Day 2	Day 3	Day 7 Post-treatment
	Pre-treatment	Post-treatment	Post-treatment	Post-treatment
PCV (%)	30.8±6.7	26.3±6.4	27.3± 6.04	29.9±6.9
Hb (g/L)	10.9 ±0.9	4.8 ± 2.5	13.6±2.21	8.04±2.6
WBC (/μL)	10.9±2.1	10.4±2.1	11.1±1.6	14.8±2.0
RBC (10 <sup>6</sup> /μL)	6.3±0.8	6.6±0.5	6.3±0.7	6.2± 0.9
PLT (/μl)	620.4± 204	194.5± 62.9	216.4±59.0	332.8±96.0

\* indicates significant difference from the pre-treatment value.

**Table 4** Summary of systemic signs observed following intramuscular administration of 2 doses of 6 mg/ kg Imidocarb dipropionate at 48 hours interval to 6 healthy dogs.

Systemic Signs	Treatment with IMDP		Duration
	First dose	Second dose	
Mild Colic	3/6	3/6	±30 minutes
Depression	2/6	2/6	2-4 hours
Anorexia	1/6	0/6	2-4 hours
Nausea	2/6	1/6	10-30 minutes
Vomiting	0/6	0/6	-

**Conclusion**

Based on the result obtained, it was concluded that treatment of diseased dogs with IMDP may induce minor

damage to hepatic profile which improves within few days post-treatment. Compared to liver, there was less affect on renal function tests. However, it is

recommended that the treated animals should be kept under surveillance as individual animal may show higher effect on RFTs.

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