EFFICACY AND ADVERSE EFFECTS OF IMIDOCARB DIPROPIONATE ADMINISTRATION TO INFECTED CATTLE WITH BABESIASIS AND HEALTHY CATTLE

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ABSTRACT

Subcutaneous injection of imidocarb dipropionate in a single theraputic dose (120 mg/100kg b.wt.) to naturally infected cattle with babesiasis resulted in disappearance of the parasites from blood smears after 24 hours post treatment.

In infected cattle, the drug displayed a gradual decrease in rectal temperature toward normal levels .On haemogram, babisiasis evoked a significant decrease in erythrocyti count, haemoglobin content and packed cell volume. The previous changes were completely disappeared, two weeks post treatment with imidocarb diprpionate. Moreover, infected cattle showed a significant increase in serum ALT , indirect bilirubin, urea levels with a significant decrease in total proteins, glucose and cholesterol levels. Two weeks post treatment, the mentioned alterations in serum constituents were completely subsided. Toxic signs were mild or absent. Hyperglycemic effect persisted throughout the experiment in both infected and healthy treated cattle.

INTRODUCTION

Babesia is an intraerthrocytic, tick transmitted protozoan disease infecting domestic and wild animals. Babesiasis cause a dramatic drop in RBCs count, PCV., Hb (**Ranatunga and Wanduragala, (1972); Al-Delaimi et al., (1989); El-Refaey, (1994); Aziz et al., (1995)**.

Acute phase of Babesiasis is accompanied by hypoproteinemia, elevation in AST, ALT, and alkaline phosphatase activities, bilirubin, glucose, creatinine, urea and uric acid were markedly increased **(Suteu and Giurgea-Iacob, (1971); Aziz et al., (1995) · Abou El-Naga, (2002)**, while cholesterol level become markedly reduced **(Elissalde et al., (1983) ; Al-Delaimi et al., (1989) ; El- Refaey, (1994)**.

Imidocarb dipropionate is a babesiocidal, anaplasmocidal drug belonging to the series of amicarbalide.According to the British Veterinary Codex (1970).Imidocarb is a 3.3-bis(2- imidazoline

2-2 Yl) carbanilide. The drug is effective against bovine babesiasis **(Addah, (1987) ; Rutter, (1990); El- Refaey, (1994), Babesia ovis (Abou El-Naga, 2002).** Imidocarb has a two compartment open model **(Abdullah and Bagot, 1983)**. It has a direct effect on the parasite, causing dilation of nuclear cisternae, karyorrhexis, cytoplasmic vaculation, inhibition of formation of food vacuoles and ribosomal diminution **(Simpson and Neal, 1980)**. Imidocarb block the entry of inositol into erythrocytes containing Babesia resulting in starvation of the parasite **(Mc- Hardy et al., 1986)**. Imidocarb had no effect on body temperature, body weight, haematology, other clinical chemistry values or gross pathology **(Abdulla and Bagot, (1984)**. In healthy goats the drug cause a transient decrease in total erythrocytic count and haemoglobin concentration, leukocytosis, an increase in MCV, MCH, MCHC. The animal started recovery from the toxic effects after eight days **(Singh, et al. (1990)**. The drug has anticholinestrase activity **(Ali et al., (1985); Michell et al., (1986); Mc- Dougald and Roberson, (1988); Singh et al., (1990)**.

The present study was carried out to determine the efficacy of imidocarb dipropionate during the treatment of cattle Babesiasis, haematological and biochemical changes that associated with the administration of the drug. Moreover its adverse effects ;if any;on both infected and healthy cattle.

MATERIALS AND METHODS

I- Drug : Imidocarb dipropionate (Imizol®) (Essex Animal Health Friesoythe, Germanay) was used in this study.

II-Animals: Twenty adults (over 2.5 years old) females, mixed breed cattle were used in this experiment. Ten of them proved to be infected with Babesia bigemina with typical clinical signs of Babesiasis. The infected animals showed fever and positive blood smears, haemoglobinurea, jaundice. The other 10 cows were apparently clinically healthy.

Diseased cattle were divided into 2 groups (each of five cattle), the first group was served as non treated controls, while the second group were injected subcutaneously with a single therapeutic dose of imidocarb dipropionate (12%) in a dose of 120 mg./100kg. b.wt.

Healthy cattle were also divided into 2 groups (each of five cattle), the first group was control non treated, meanwhile the second was treated with imidocarb dipropionate (120 mg/ 100kg b. wt.). Results of treated cattle were compared with untreated controls.

Efficacy of imidocarb dipropionate :

Rectal temperature was recorded before and at 1, 2, 3 days post administration of the drug. Blood films for detection of the parasite were performed according to **Coles, (1986)**.

Haematological studies : Jugular venous blood samples of about 5 ml were obtained from each animal in a clean bottle containing 10 mg. EDTA as anticoagulant.

Double improved Neubaur's haemocytometer was used for counting erthrocytes (RBCs),total leucocytic count according to **Wintrobe, (1967)**, while haemoglobin concentration (Hb) was determined using Sahli's- haemometer **Schalm et. al., (1975)**. Wintrobe haematocrit tubes were used for determining the packed cell volume (PCV)by centrifugation at 3000 r.p.m. for 45 minutes **Wintrobe, (1967)**.

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), were calculated according to **Schalm et. al., (1975)**. Haematological examination was made before treatment and after 24 hours, one and two weeks post treatment.

Biochemical studies :another blood sample was collected without anticoagulant, left to clot in slant position at room temperature and stored over night in a refrigerator at 4C0 , then samples were centrifuged at 3000 r.p.m. for 20 minutes for clear serum separation. Some biochemical parameters(AST,ALT, glucose, total protein, total bilirubin, cholesterol, creatinine ,urea and uric acid)were measured according to **Reitman and Frankel (1957); Emerson, (1943); Doumas, (1975); Malloy and Evelyn (1937); Burchard, (1980); Folin, (1934); Fawcett and Scott (1960); James and White (1971)** respectively using Spectrophotometer (Spectrronic 20D, Milton Ray company) and kits(Bio-Adwic).

Statistical analysis : Student (t) test was used **(Snedecor & Cochran, 1981)**.

RESULTS

Efficacy of imidocarb dipropionte on infected cattle after a single s/c injection(120 mg/ 100kg b. wt.) :

a) Body temperature : decreased from 41.12C° on day zero tell reaching 38.3C° on the second day and 38.2C° on the third day post treatment (Table 1).

b) Blood smears: were negative, 24 hours after drug administration.

c) Haematological and biochemical changes :

Haematological changes:

Babesiasis produced a significant decrease in erthrocytic count ($p<0.01$), haemoglobin content($p<0.001$), packed cell volume ($p<0.001$).

One day post treatment a significant increase in MCHC ($p<0.05$) was recorded.

One week post treatment MCV was significantly decreased ($p<0.05$) , while MCHC was significantly increased ($p<0.05$). Two weeks post drug administration , the erythrogram of infected cattle become similar to the erythrogram of the control.

No significance changes were reported on the total leucocytic count.(Table 2).

Biochemical changes:

Babesiasis evoked a significant increase in serum ALT , urea ($p<0.001$), indirect bilirubin ($p<0.05$) levels with a significant decrease in cholesterol, glucose, creatinine, uric acid ($p<0.001$) and total protein ($p<0.05$)levels. The previous changes in serum constituent were completely subsided two weeks post treatment with imidocarb dipropionate (Table 3).

Haematological and biochemical changes induced by imidocarb dipropionate (120mg/ 100 kg b. wt.) in healthy cattle :

a) Haematological changes :

The obtained results revealed an increase in erythrocytic count ($p<0.01$), haemoglobin content ($p<0.05$) with a significant decrease in mean corpuscular volume ($p<0.01$), mean corpuscular haemoglobin($p<0.05$) on day 7 after administration. On day 14 after drug administration, a significant increase of erythrocytic count ($p<0.01$) was recorded ,while the mean corpuscular volume($p<0.01$), mean corpuscular haemoglobin($p<0.05$) were significantly decreased .Packed cell volume values were slightly increased one day post treatment and continued to increase, one and two weeks post treatment. Total leucocytic counts fluctuated toward normality from one day to two weeks post treatment (Table 4).

b) Biochemical changes :

The S/C administration of imidocarp dipropionate caused a significant decrease in serum aspartate aminotransferase enzyme (AST) activity, creatinine, urea ($p<0.001$), serum cholesterol levels ($p<0.01$), ($p<0.001$),($p<0.001$) respectively, uric acid($p<0.01$), ($p<0.01$), ($p<0.001$) at one day, one and two weeks post treatment respectively, total bilirubin($p<0.01$),indirect bilirubin ($p<0.001$) at two weeks post treatment, compared to control values. (Table 5).

A significant hyperglycemia ($p<0.001$) was appeared at one day, one and two weeks post treatment (Table 5).

Toxicological studies of imidocarb :

Imidocarb dipropionate in a single subcutaneous injection dose (120mg/100kg b. wt.) resulted in a local reactions in some treated cattle which involved a slight swelling at the injection site after 24 to 48 hours which regressed without trace. Rarely animals were seen to show a systemic reactions with consistent features of salivation, panting, a soft cough and restlessness, these reactions lasted for 30 to 45 minutes.

No subsequent unwanted effects on the pregnant cattle were observed.

DISCUSSION

Babesiasis could be controlled by a variety of measures including chemotherapy with antibabesial drugs and preventing infection by maintaining an adequate tick control program to keep cattle free from tick infestation **(Jones et al., 1977)**.

In the present investigation, it has been demonstrated that imidocarb diprpionate in a single therapeutic dose (120mg/100kg b. wt.) given subcutaneously is highly effective against Babesia infection . where the blood parasites were completely disappeared from the peripheral blood one day post treatment. Many authors recorded the effectiveness of imidocarb **(Higgins, (1981) ; Michael and Refail, (1982); Mc Hardy et al., (1986); El- Refaey, (1994) ; Mahmoud, (1996) ; Ignasi et al., (2000) and Abou-El-Naga, (2002)**. Babesiasis elicited a significant decrease in red blood corpuscles, haemoglobin and packed cell volume. Similar results have been documented in cattle **(Wright, (1973); El-Refaey, (1994) Aziz et al., (1995) and** in horses **(Al-Delaimi et al., (1989)**. These alterations in blood picture were completely disappeared, two weeks post treatment. These results are in accordance with those obtained in cattle **(Wright, (1973); Rutter, (1990); El-Refaey, (1994)**, sheep and goats **(Ali et al., (1985); Mc Hardy et al., (1986); Abou El-Naga, (2002)** and dogs **(Michael et al., (1973)** .

In healthy treated cattle, a significant increase in erythrocytic count, haemoglobin concentration and in haematocrite were recorded compared to the control values at 7 days and 14 days after drug administration. These results were not in agreement with that reported by **Singh et al., (1990)** in healthy goats who reported a significant decrease in RBCs count and Hb.%. The variation in haematologic values may be due to imbalanced nutrition or other stress factors.

Concerning the effects of imidocarb (120mg/100 kg b. wt.) on total leucocytic count, no changes of significance were reported.This result was consisted with that reported by **(Ranaiunga and Wanduragala, (1972); Wright, (1973); El-Refaey, (1994)** in infected cattle. In healthy treated cattle, leukocyte count fell initially but reached maximum values at 14 days post treat-

ment .This result was not in agreement with that reported by Singh et al.,(1990) in healthy goat, where the drug caused leukocytosis.

Serum Alanine amino transferse (ALT), indirect bilirubin were significantly elevated in infected cattle, indicating liver affection. Similar results were reported by **Fowler et al., (1972); Abuzina, (1989); Al-Delaimi et al., (1989); El-Refaey, (1994); Yeruham et al., (1998a and b); Abou-El-Naga , (2002)**.

Total serum protein levels were markedly reduced from normal values, however contro verse results were recorded, so it could decrease **(Suteu and Giurgea, (1971); El-Refaey, (1994) ; Aziz et al., (1995)** and not significantly decrease in infected sheep (Abou- El-Naga ,(2002).

Hypocholestrolemia and hypoglycemia were scored in infected cattle in comparison to control animals. Cholesterol level was said to be either increased **(Muley et al., 1980); Abuzina,(1989); Aziz et al., (1995) and Abou- El-Naga, (2002) or decreased (Elissalde et al.,1983) ; El-Delaimi et al., (1989)**. Cholesterol may increase during exposure to heat, exercise and decrease after exposure to cold **(Farahat et al.,1995)**, this indicates that cholesterol is modulated according to stress which induce changes in plasma and liver fatty acid composition **(Huang et al., 1990)**.

After drug administration, hyperglycemia was resulted in both infected and healthy cattle, However, contro verse results were recorded, so it could be increased due to infection **Aziz et al., (1995) ; Abou-El-Naga, (2002)**.

The hyperglycemic effect immediately occurred post treatment thought to be a result of alpha 2 adrenoceptor mediated depression of insulin release from pancreatic beta cells. Increased glucose level post treatment may be due to enhanced glycogenolysis secondary to sympathetic stimulation or due to under endocrine control and in this respect the role of adrenaline and cortisol are S particularly important.

An important increase in urea value was found in infected animals , similar results were recorded by **Fowler et al., (1972); Al-Delaimi et al., (1989); El- Refaey, (1994) ; Yeruham et al., (1998a &b); Abou-El-Naga,(2002)**.

Haemoglobin released from fragmented erythrocytes due to parasite multiplication pass through the kidney causing nephrotoxic effects and electrolytes imbalance (Blood and Radostitis 2000). In healthy treated cattle the drug failed to induce any significant change in serum urea nitrogen, this result was in agreement with that reported by **Abdulla and Bagot,(1984) and Singh et al., (1990)**.

Regarding to toxicity of imidocarb, our results showed a local and systemic reactions lasted

for 30 to 45 minutes. Similar observations were recorded by **Callow and Mc Gregor (1970); Haigh and Hagans, (1974) Ali et al.,(1985); Michell et al., (1986); Mc Dougald and Robertson, (1988) ; Singh et al., (1990).**

The proper evaluation of the treatment trials was observed by improvement in the studied haematological and biochemical parameters adversely affected by the infection .Improvement in the liver and kidney functions were indicated by significant decrease in the levels of serum enzymes, cholesterol, total, direct and indirect bilirubin and creatinine in additional improvement in serum urea level . Our evaluation is supported by **Manston and Allen ,(1981)** who recorded several documents led to the hypothesis that haematologic values reflect the balance between nutritional input, efficiency of metabolic through put and requirements of productive output . The appearance of variation in Hb% ,haematocrit value and glucose concentration ensure that these parameters appeared to be sensitive indicators of stress in infected cattle .

The effect of the imidocarb dipropionate on carbohydrate, fat , and protein metabolism are not completely understood, but the drug increases blood sugar levels and normally reduces the metabolic use of carbohydrates, also this study demonstrates that the margin of safety for imizol administration to cattle at 120mg/100kg. body weight subcutaneously is adequate for its intended use.

Table (1) : The effect of s/c injection of imidocarb dipropionate (120mg / 100kg .b.wt) on rectal temperature of cattle infected with Babesia bigemina (Mean ± S.E.) (n = 5)

Animal	Rectal temperature(C°)
Control	37.85 ± 0.175
Before treatment	41.12 ± 0.225***
One day post . treatment	38.88 ± 0.203*
Second day post . treatment	38.30 ± 0.095
Third day post treatment	38.24 ± 0.102

* P < 0.05 *** P <0.001

Table (2): Haematological changes induced by a single s/c injection of imidocarb dipropionate (120mg / 100kg b.wt) in Babesia bigemina infected cattle. (Mean ± S.E.) (n.=5) .

Animal condition	RBCs (10^6/Cu mm)	Hb (gm%)	PCV (%)	MCV cuu	MCH uug	MCHC %	WBCs 10^3/Cumm
Control (non infected ,non treated)	5.15 ± 0.27	7.16 ± 0.32	39.80 ± 1.66	78.53 ± 6.389	14.12 ± 1.123	18.033 ± 0.677	9.77 ± 1.16
Infected (before treatment)	2.15 ± 0.50**	3.14 ± 0.39***	12.20 ± 2.2***	60.98 ± 7.143	16.55 ± 2.807	27.733 ± 4.658	7.77 ± 0.86
One day post treatment	3.79 ± 0.50	5.34 ± 0.43*	19.60 ± 1.72***	55.44 ± 8.993	15.61 ± 3.01	28.271 ± 3.657*	7.78 ± 0.31
One week post treatment	5.43 ± 0.55	6.42 ± 0.588	26.20 ± 2.06***	49.74 ± 5.113*	12.22 ± 1.422	24.881 ± 2.454*	8.20 ± 1.34
Two weeks post treatment	7.60 ± 0.16***	7.56 ± 0.43	33.00 ± 3.21	42.56 ± 3.446**	9.898 ± 0.507*	23.403 ± 1.494*	9.40 ± 1.13

*P < 0.05 ** P < 0.01 *** P <0.001

Table (3) : Biochemical changes induced by a single s/c injection of imidocarb dipropionate (120 mg/100kg. b. wt.) in the serum of bigemina infected cattle. (Mean+ S. E.) (n=5)

Parameters / Groups	AST	ALT	Total protein	Cholesterol	Glucose	Bilirubin			Creatinine	Urea	Uric acid
						Tot. bilirubin	Direct bilirubin	Indirect bilirubin			
	I.u/L	I.u/L	g/100ml	mg%	mg%	mg%	mg%	mg%	mg/100 ml	mg/100ml	mg/100ml
Control healthy (non treated)	45.5 ± 0.532	4.96 ± 0.358	8.92 ± 0.432	221 ± 4.483	85.51 ± 1.83	0.466 ± 0.022	0.166 ± 0.032	0.299 ± 0.019	1.61 ± 0.064	35.68 ± 0.241	0.577 ± 0.012
Infected (before treatment)	24.6 ± 0.509***	8.30 ± 0.145***	7.10 ± 0.163*	173.6 ± 0.819***	27.9 ± 0.356***	0.472 ± 0.007	0.115 ± 0.015	0.357 ± 0.004*	0.668 ± 0.0115***	52.4 ± 0.509***	0.347 ± 0.003***
One day post treatment	39.42 ± 3.055	8.20 ± 0.094***	8.10 ± 0.156	176.62 ± 3.836***	65.8 ± 0.078***	0.369 ± 0.010**	0.111 ± 0.028	0.258 ± 0.010	0.784 ± 0.009***	44.44 ± 1.00***	0.414 ± 0.022**
One week post treatment	35.08 ± 1.974**	4.40 ± 0.155	8.00 ± 0.101	128.46 ± 2.981***	85.76 ± 0.663	0.442 ± 0.015	0.184 ± 0.029	0.238 ± 0.012*	0.792 ± 0.012***	23.72 ± 0.954***	0.638 ± 0.027
Two weeks post treatment	23.2 ± 0.807***	4.18 ± 0.392	8.14 ± 0.128	216.80 ± 4.726	110.18 ± 1.664***	0.314 ± 0.021**	0.143 ± 0.03	0.171 ± 0.023**	0.456 ± 0.016***	28.6 ± 1.695**	0.491 ± 0.013**

* p < 0.05 ** p < 0.01 *** p < 0.001

Table (4): Haematological changes induced by a single s/c injection of imidocarb dipropionate (120mg / 100kg b.wt) in healthy cattle. (Mean ± S.E.) (n = 5)

Animal condition	RBCs (10^6/Cumm)	Hb (gm%)	PCV (%)	MCV cuu	MCH uug	MCHC (%)	WBCs 10^3/Cumm
Control (non infected ,non treated)	5.15 ± 0.27	7.16 ± 0.32	39.80 ± 1.66	78.53 ± 6.389	14.12 ± 1.123	18.033 ± 0.677	9.77 ± 1.16
One day post treatment	6.74 ± 0.73	7.32 ± 0.35	41.00 ± 1.52	63.175 ± 5.343	11.277 ± 1.015	17.890 ± 0.782	7.62 ± 1.17
One week post treatment	8.81 ± 0.66**	8.18 ± 0.22*	42.20 ± 1.46	48.509 ± 2.234**	9.444 ± 0.586*	19.476 ± 0.843	12.63 ± 1.19
Two weeks post treatment	9.01 ± 0.74**	8.20 ± 0.26	43.40 ± 1.44	49.076 ± 3.023**	9.304 ± 0.687*	18.929 ± 0.576	10.17 ± 0.67

* P < 0.05 ** P < 0.01 *** P <0.001

Table (5): Biochemical changes induced by a single s/c injection of imidocarb dipropionate (120 mg/kg. b. wt.) in the serum of healthy cattle . (Mean+ S. E.) (n=5)

Parmeters / Groups	AST I.u./L	ALT I.u./L	Total protein g/100ml	Cholesterol mg%	Glucose mg%	Bilirubin			Creatinine mg/100 mL	Urea mg/100ml	Uric acid mg/100ml
						Tot. bilirubin mg%	Direct bilirubin mg%	Indirect bilirubin mg%.			
Control healthy (non treated)	45.5 ± 0.532	4.96 ± 0.358	8.92 ± 0.432	221.00 ± 4.483	85.51 ± 1.83	0.466 ± 0.022	0.166 ± 0.032	0.299 ± 0.019	1.61 ± 0.064	35.68 ± 0.241	0.577 ± 0.012
One day post treatment	21.80 ± 0.860***	3.96 ± 0.104	8.58 ± 0.510	159.66 ± 4.297**	236.788 ± 4.197***	0.491 ± 0.069	0.126 ± 0.046	0.365 ± 0.069	0.714 ± 0.042***	28.80 ± 0.340***	0.418 ± 0.023**
One week post treatment	17.40 ± 1.435***	4.02 ± 0.541	10.22 ± 1.046	170.54 ± 4.606***	227.90 ± 4.885***	0.434 ± 0.005	0.190 ± 0.019	0.245 ± 0.023	0.652 ± 0.029***	27.40 ± 0.841***	0.414 ± 0.029**
Two weeks post treatment	23.00 ± 0.707***	4.03 ± 0.081	9.15 ± 0.491	179.42 ± 2.115***	185.52 ± 2.245***	0.262 ± 0.045**	0.200 ± 0.036	0.066 ± 0.023***	0.80 ± 0.011***	25.6 ± 0.260***	0.452 ± 0.012***

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

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الملخص العربى

الكفاءة والآثار الجانبية لعقار الأميدوكارب داى بروبيونيت فى الأبقار المصابة بمرض البابيزيا والأبقار السليمة

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اقد أجريت هذه الدراسة من أجل تقييم تأثير أملاح الأميدوكارب داى بروبيونيت (الأميزول) فى علاج مرض البابيزيا وهو أحد أمراض طفيليات الدم فى الأبقار والذى يحدث آثار سلبية فى صورة الدم وبعض وظائف الكبد والكلى.

ولقد تبين من الدراسة أن إعطاء أملاح الأميدوكارب داى بروبيونيت فى الأبقار المصابة بمرض البابيزيا أدى إلى إختفاء الطفيل من الدم ثم عودة تدريجية فى درجات الحرارة لمعدلاتها الطبيعية وتحسن سريع فى عدد كرات الدم الحمراء والهيموجلوبين ومكداس الدم.

كما عادت وظائف الكبد والكلى إلى مستواها الطبيعى بعد إسبوعين من العلاج واتضح أيضاً أن الآثار الجانبية لهذا العقار سواء فى الأبقار المصابة أو السليمة كانت بسيطة ومؤقتة حيث اختفت بعد ٤٨ ساعة من العلاج.

إلا أن العقار كان له أثر فى زيادة نسبة السكر فى الدم عن معدلاتها الطبيعية واستمرت هذه الزيادة حتى نهاية التجربة فى كل من الأبقار المصابة والسليمة.