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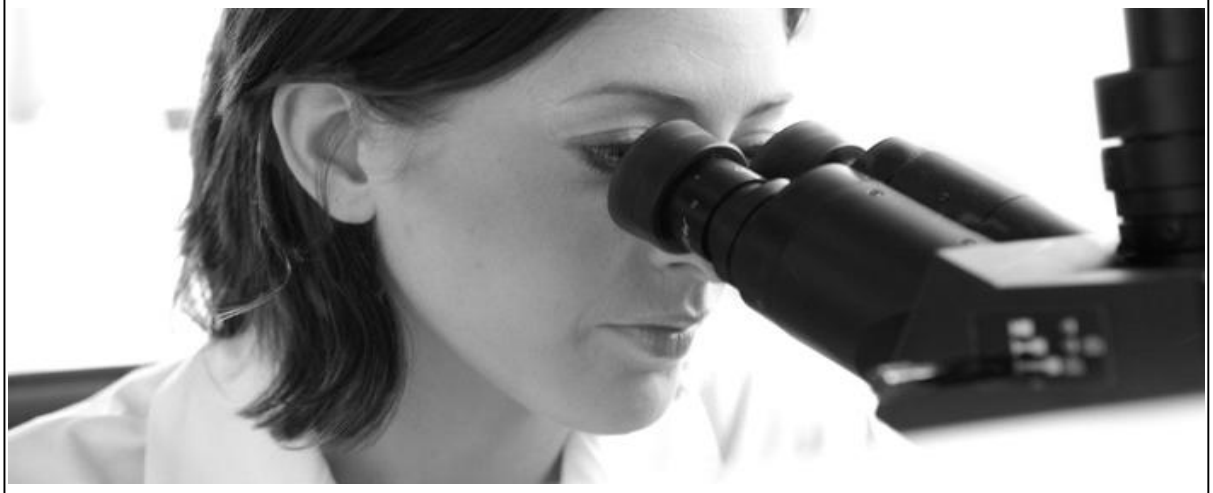

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RESEARCH ARTICLE

THE ANTI-COAGULANT BROMADIOLONE IMPACT ON HAEMATOLOGY AND BIOCHEMICAL CHANGES IN *BANDICOTABENGALENSIS* (*GRAY AND HARDWICKE*)

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ABSTRACT

Bandicotabengalensis Gray and Hardwicke ratsare reported to beserious pests destroying crops, fruit gardens, orchards and stored food grains. Rodents are also responsible for spreading fatal diseases like *Plague, Leptospirosis, Murine typhus*. In the present exploration the bromadioloneis a second generation anticoagulant rodenticide was tested on *Bandicotabenglensis*rats for its effects on blood (haematological) and biochemical changes at varied time intervals. The rats were grouped in to 7 groups of 6 animals in each group. Test animals were fed with bromadiolone (0.005%) for 6 days in no-choice test in the form of cake bait (mixed in plain WSO)at doses 100mg/kg/BW of different time interval 12 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs. The result revealed that a significantaltered hematological parameters (RBC, WBC, and Platelet), oxidative stress markers (TBARS, LOOH, PC and CD) and antioxidant status (enzymatic and non-enzymatic) in rats. The observed information demonstrated that the oral supplementation of bromadiolonemay led to damage the haematological and biochemical changes in *B. bengalensis* rat. Therefore, it can be suggested that the use of bromadioloneas an effective rodenticide to control this pest in the selected agricultural fields.

INTRODUCTION

No country in the world is free from quantitative and qualitative losses inflicted by rodents. However, the losses are more pronounced in the tropical countries where rodents play a significant role in retarding agricultural production through damage at both pre and post harvest stages (Chattopadhyay *et al*., 2010; Singla and Babbar, 2010, 2012; Gogoi and Borah, 2013). The lesser bandicoot rat, *Bandicotabengalensis* (Gray and Hardwicke) is the most predominant rodent pest species in agriculturaland commensal situations (Musser and Carleton, 2005).Withyield losses of 5-15% in most countries (Palis *et al*., 2007; Singleton,2003). There are reports of rodentscausing 6-8% loss in rice (*Oryzasativa L*.) and 10-12% in wheat*(Triticumaestivum L.)* in India (Chattopadhyay *et al*., 2010; Santraand Manna,2008). The highlighted the fact thatrodents account for 11.8% in rice, 12.4% in wheat and 22.4% insugarcane (*Saccharamofficinarum L*.) of total yield loss attributed to biotic and abiotic factors. The United States has an estimated approximately 1.25 billion rats are in the world wide and they cause at least$19 billion crop loss damage due to rats at every year. Worldwide, there are 3.5 million rats are born every day (Addison,2012).Rodents also pose a serious health risk as transmitters of several diseases to humanand domestic animals such as *Leptospirosis, Saslmonellosis, Trichinosis, Hantavirus pulmonary syndrome, Hantavirus renal syndrome*, Argentine hemorrhagic fever and lymphocytic choriomeningitis (Castillo *et al*., 2003).

Blood analyses are useful, widely used tools that aid in the diagnosis and monitoring of animal health and disease, and in the differentiation of physiologic processes. The blood of rodents (*Bandic otabengalensis*) contains nucleated erythrocytes, nucleated thrombocytes, heterophils, eosinophils, basophils, lymphocytes, and monocytes. Hematology is used to detect conditions related to these cells, such as anemia, inflammatory diseases, parasitemias, hematopoietic disorders, and haemostatic alterations (Terry, 2006).

Bromadiolone (3- [3- (40 - bromobiphenyl- 4)-3-hydroxy-1-phenylpropyl]-4 hydroxycoumarin)(Fig.1) Second generation anticoagulant rodenticide, which provide an immediate solution to the rodent problem, form the major component of rodent control strategies in India(Neeraj *et al*.,2013).The only practical and feasible method to bring down rodent population effectively over a large area is by the use of rodenticides Firstly, to evaluate the effect haematologicalat varied time intervals viz., 12hrs, 24hrs, 48hrs, 72hrs, 96hrs, and 120 hrs. However, the impact of bromadiolone on hematological and biochemical parameters of *B. bengalensis* erythrocytes have not yet been studied. Thus give the inspiration to evaluate the effects of bromadiolone on blood biochemical markers changes

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in *B. bengalensis* rat. Animals were fed with bromadiolone in the form of bait at 12hrs, 24hrs, 48hrs, 72hrs, 96hrs, and 120hrs, hrs at different time intervals.

MATERIALS AND METHODS

Chemicals

Bromadiolone (C_{30} H_{23} BrO_4,) Fig.1, is a second generation anticoagulant and is commercially available under the trade name "MooshMoosh". Bromadiolone wax cake is directly palatable to the test animal. All other chemicals and solvents were of certified analytical grade and purchased from S.D. Fine Chemicals, Mumbai or Himedia Laboratories Pvt. Ltd., Mumbai, India. Reagent kits were obtained from span Diagnostics, Mumbai, India

Animals and diet

Male *B. bengalensis*(150-250g weight) were live-trapped from crop fields in and around Sethur Village, Thirunallar Commune, Pondicherry (UT),and India. In the laboratory, rats were acclimatized individually in cages (36 × 23 ×23) with food and water provided *ad libitum* for 6 days before the commencement of the experiment. The rats were kept in a room with a 12 h light/12 h dark photoperiod and temperature of 24°–26 °C.

Experimental design

In the present study, mature and healthy male rats were screened for signs of no diseases; Totally 42 rats were used in this study and they were divided into 7 groups of 6 animals each. At every baiting station, bait weighing 100 gm was offered to the rodents (Mixed with wheat, powdered sugar and groundnut oil at a ratio of 96:2:2.) and was replenished with fresh bait daily for a period of 6 days. Weighing of the baits was accomplished with a "weighing balance" after an allotted hour interval, and the quantity of the bait consumed was recorded. The LD_{50} value of bromadiolone was 1.125 mg/kg of 100gm of BDL (0.005%) (Meister *et al*., 1984).

Group 1: Served as control fed with normal diet and water.

Group 2: Rats were fed with bromadiolone (100g/kg BW mixed with wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2.) for 12 hrs.

Group 3: Rats were fed with bromadiolone (100g/kg BW mixed with wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2.) for 24hrs.

Group 4: Rats were fed with bromadiolone (100g/kg BW mixed with wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2.) for 48 hrs.

Group 5: Rats were fed with bromadiolone (100g/kg BW mixed with wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2.) for 72 hrs.

Group 6: Rats were fed with bromadiolone (100g/kg BW mixed with wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2.) for 96 hrs.

Group 7: Rats were fed with bromadiolone (100g/kg BW mixed with wheat, powdered sugar and groundnut oil (WSO bait) at a ratio of 96:2:2.) for 120 hrs.

At the end of each stage, after overnight starvation, the rats were anesthetized with chloroform choked in cotton kept inside the bottle. Blood was collected by heart puncture and immedia telytransferred into tubes containing serum separator or potassium-EDTA. After 45 min blood samples incubation in room temperature, serum was separated by centrifugation at 3000xg for 10 min at 4^0C.

Haematological analysis

The procedure followed was based on the instruction manual of haematology auto analyser SFRI. France. (H 18 LIGHT). Total red blood cell (RBC) count ($x10^3$6/uL), haemoglobin (HCT; %), total number of white blood cell (WBC) or leukocytes ($x10^3$/uL), lymphocyte count (LYM; $x10^3$/uL), platelet (PLT) count ($x10^3$/uL) were assessed. Mean corpuscular volume (MCV; fL), mean corpuscular haemoglobin (MCH; pg), mean corpuscular haemoglobin concentration (MCHC; fL) were also calculated.

Isolation of erythrocytes and erythrocyte ghost membranes

Erythrocytes and their ghost membranes were prepared by (Dodge *et al.* 1963) and (Fairbanks *et al.* 1971) with slight modifications. Packed cells were washed with saline. Packed cells were washed with Tris-buffer, 0.31 M, and pH 7.4 and used for biochemical estimations. Another packed cell was used for hemolysis by adding hypotonic 5 mm phosphate buffer (pH 8.0) with the addition of 1 mm EDTA. After 4–6 h, the erythrocyte ghosts sedimented by centrifugation at 12000 rpm for 45 min at 4–6 ◦C. The hemolysate was used for the antioxidant assay. The erythrocyte membrane pellets were suspended in 0.02 M Tris-buffer (pH 7.2) and used for various biochemical assays.

Estimation of erythrocytes membrane lipid peroxidation

Lipid peroxidation in terms of thiobarbituric acid reactive substances was measured according to the method of (Esterbauer and Cheeseman, 1990) and the Protein carbonyl levels were measured according to method described by (Reznick and Packer, 1994). Measurement of conjugated diens in erythrocytes was according to (Konings, 1984).

Determination of non-enzymatic antioxidants

Reduced glutathione (GSH) content was estimated according to the method of (Beutler *et al.* 1963) and expressed as µmoles/g Hb. Total sulfhydryl groups (TSH) were measured after reaction with dithionitrobis benzoic acid, using the method of (Ellman, 1959). Concentrations of vitamins C and E were measured following the methods of (Omaye *et al.* 1979) and (Desai, 1984), respectively.

Assay of enzymatic antioxidants

Erythrocytes from the second tube were lysed by four-fold dilution with H2O, followed by repeated freezing–thawing cycles. SOD (U/g Hb) activity was estimated according to the method described by (Misra and Fridovich, 1972). CAT (U/g Hb), activity was determined using the method described by (Aebi, 1984), by measuring hydrogen peroxide decomposition at 240 nm. GPx (U/g Hb) activity was assayed using the method described by (Flohe and Gunzle, r1984), by the subsequent oxidation of NADPH at 240 nm with t-butyl-hydroperoxide as substrate. The values were expressed in units per gram of hemoglobin. GR activity in erythrocytes was assayed by the methods of Goldberg and Spooner (1983). The GR activity in erythrocytes has been expressed as nM NADPH oxidized to NADP/ g of Hb/min. Glutathione-S-transferase (GST) measured by (Buetler, 1984).

Statistical analysis

The data were presented as mean±S.D and were analysed using statistical package for social sciences (SPSS) version 16.0. The Student's t-test was used to compare mean values of haematology parameters measured between exposed and unexposed groups. The criterion for significant was set at *p<0.05*.

RESULTS

Effect of bromadiolone on erythrocytes

Table 1 shows the effect of bromadiolone on erythrocytes of control and experimental rats. The erythrocytes (RBC) and other subsequent significantly (P<0.05) decreased from normal level in bromadiolone treated rats (24hrs-120hrs) when compared with control rats. The group II rats also show a slight change in the RBC levels when compared with control.

Effect of bromadiolone on erythrocytes indices

The effect of bromadiolone on erythrocytes indices of control and experimental rats were shown in table 2. The levels of MCV, MCH and MCHC significantly (P<0.05) decreased in bromadiolone treated rats (24hrs-120hrs) when compared with the control rats. The rat treated with bromadiolone for 12 hrs did not show significant changes compared with control.

Effect of bromadiolone on Leukocytes

Table 3 shows the effect of bromadiolone on leukocytes of control and experimental rats. The leukocytes (WBC) and other subsequent parameters significantly (P<0.05) decreased in bromadiolone treated rats (24hrs-120hrs) when compared with control rats. The group II rats did not show any changes in the WBC levels when compared with control.

Effect of bromadiolone on blood coagulation parameters

Table 4 depicts the effect of bromadiolone on thrombocytes of control and experimental rats. The rat treated with bromadiolone for 24-120 hrs revealed a significant decrease thrombocytes were observed when compare with control rats. The rat fed with bromadiolone for 12 hrs also observedslight changes compared with control.

Effect of bromadiolone on lipid peroxidation

Table 5 shows the changes in the levels of lipid peroxidation markersof erythrocytes in the control and experimental animals. The rats fed with BDL for 48hrs, 72hrs, 96hrs and 120 hrs, showed a significant increase *(P<0.05)* levels of TBARS, LOOH, PCC and CD when compared with control rats.

Table 1 Effect of bromadiolone on Erythrocyte Indies in control and experimental rats

S. No	Blood Parameters	Group I Control	Group II 12 hrs.	Group III 24 hrs.	Group IV 48 hrs.	Group V 72 hrs.	Group VI 96 hrs.	Group VII 120 hrs.
1	Hb (g/dL)	15.89±0.523^{a}	15.23±0.340^{b}	14.53±1.372^{c}	14.11±1.120^{d}	13.1±1.328^{e}	11.20±1.43^{f}	9.02±1.218^{g}
2	RBC (10^{6}/uL)	8.92±0.467^{a}	8.87±0.412^{b}	8.80±0.347^{c}	8.75± 0.302^{d}	7.76±0.293^{e}	6.20±0.271^{f}	5.10±0.153^{g}
3	RDW (fL)	52.2±2.347^{a}	50.23±2.332^{b}	42.12±2.124^{c}	24.12±1.761^{d}	19.24±1.23^{e}	15.1±1.212^{f}	11.24±1.021^{g}
4	HCT (%)	52.01±1.328^{a}	51.67±1.301^{b}	49.43±1.124^{c}	44.32±1.101^{d}	35.0±0.771^{e}	19.12±0.47^{f}	17.32±0.411^{g}

Values are given as mean±SD from six rats in each group.$^{a–g}$ Values with different superscript letter (a–g) in the same row differ significantly at p < 0.05 (DMRT).

Table 2 Effect of bromadiolone on Erythrocytes MCV, MCH, and MCHC of control and experimental rats

S. No	BloodParameters	Group I Control	Group II 12 hrs.	Group III 24 hrs.	Group IV 48 hrs.	Group V 72 hrs.	Group VI 96 hrs.	Group VII 120 hrs.
1	MCV (fL)	59.72±0.961^{a}	58.48±0.952^{b}	55.65±0.894^{c}	48.87±0.643^{d}	35.79±0.553^{e}	31.92±0.552^{f}	23.1±0.553^{g}
2	MCH (pg)	21.12±0.853^{a}	20.97±0.811^{b}	20.23±0.789^{c}	17.54±0.612^{d}	17.1±0.571^{e}	16.23±0.548^{f}	11.1±0.521^{g}
3	MCHC(g/dL)	35.07±0.574^{a}	34.89±0.563^{b}	31.05±0.512^{c}	26.94±0.486^{d}	21.70±0.381^{e}	16.75±0.349^{f}	14.12±0.55^{g}

Values are given as mean±SD from six rats in each group.$^{a–g}$ Values with different superscript letter (a–g) in the same row differ significantly at p < 0.05 (DMRT).

Table 3 Effect of bromadiolone on Leukocytes of control and experimental rats

S. No.	Blood Parameters	Group I Control	Group II 12 hrs.	Group III 24 hrs.	Group IV 48 hrs.	Group V 72 hrs.	Group VI 96 hrs.	Group VII 120 hrs.
1	WBC (10^{3}/uL)	10.95±0.752^{a}	10.90±0.721^{b}	10.12±0.705^{c}	10.1±0.698^{d}	7.52±0.520^{e}	5.80±0.463^{f}	3.10±0.352^{g}
2	Lymphocyte(10^{3}/L)	7.12±0.837^{a}	7.11±0.821^{b}	7.2±0.789^{c}	6.89±0.711^{d}	6.60±0.666^{e}	4.0±0.538^{f}	5.10±0.453^{g}
3	Neutrophils	20.31±0.38^{a}	20.11±0.20^{b}	19.02±0.21^{c}	15.01±0.28^{d}	12.12±0.25^{e}	9.76±0.23^{f}	6.23±0.13^{g}
4	Monocyte	6.13±0.512^{a}	6.09±0.509^{b}	6.21±0.487^{c}	5.65±0.435^{d}	4.31±0.365^{e}	3.32±0.265^{f}	1.58±0.211^{g}
5	Eosinophil	4.12±0.489^{a}	4.23±0.482^{b}	4.34±0.469^{c}	4.53±0.432^{d}	3.56±0.421^{e}	3.12±0.378^{f}	3.02±0.127^{g}
6	Basophils	0.58±0.341^{a}	0.51±0.321^{b}	0.46±0.311^{c}	0.55±0.387^{d}	0.59±0.412^{e}	61.1±0.423^{f}	64.12±0.432^{g}

Values are given as mean±SD from six rats in each group. $^{a–g}$Values with different superscript letter (a–g) in the same row differ significantly at p < 0.05 (DMRT).

Table4 Effect of bromadiolone on Thrombocytes indices in control and experimental rats

S. NO	Blood Parameters	Group I Control	Group II 12 hrs.	Group III 24 hrs.	Group IV 48 hrs.	Group V 72 hrs.	Group VI 96 hrs.	Group VII 120 hrs.
1	PLT(x10^3/uL)	1122±12.75^a	1120±12.13^b	1098±12.05^c	985±11.89^d	678±11.03^e	336±10.98^f	298±10.29^g
2	PDW (fL)	15.23±0.675^a	15.01±0.671^b	13.92±0.612^c	9.76±0.556^d	6.89±0.459^e	5.69±0.411^f	4.19±0.298^g
3	MPV (fL)	15.98±0.458^a	15.29±0.435^b	14.12±0.412^c	12.23±0.389^d	7.89±0.212^e	6.92±0.201^f	5.19±0.194^g
4	P-LCR (%)	18.39±0.698^a	18.11±0.675^b	16.23±0.617^c	14.45±0.611^d	9.38±0.459^e	5.12±0.341^f	2.78±0.213^g

Values are given as mean±SD from six rats in each group. a–g Values with different superscript letter (a–g) in the same row differ significantly at $p < 0.05$ (DMRT).

Table 5 Effect of Bromadiolone on erythrocyte lipid peroxidation markers in control and experimental rats

S.No	Erythrocyte Lipid Peroxidation Markers	Group I CONTROL	Group II 12 hrs.	Group III 24 hrs.	Group IV 48 hrs.	Group V 72 hrs.	Group VI 96 hrs.	Group VII 120 hrs.
1	TBAR	1.17±0.32^a	1.20±0.36^b	1.38±0.49^c	1.69±0.61^d	2.11±0.93^e	3.25±1.24^f	4.08±2.31^g
2	PC	1.76±0.34^a	1.89±0.43^b	2.14±0.78^c	3.23±1.02^d	4.13±1.54^e	5.12±2.21^f	5.53±2.89^g
3	CD	4.12±1.03^a	4.21±1.12^b	5.09±1.43^c	6.13±2.09^d	7.67±2.46^e	8.04±3.11^f	8.78±3.98^g
4	LOOH	12.03±2.12^a	12.11±2.16^b	13.45±2.35^c	13.86±2.67^d	14.34±3.04^e	15.03±3.87^f	17.49±5.02^g

Values are given as mean±SD from six rats in each group. $^{a–g}$ Values with different superscript letter (a–g) in the same row differ significantly at $p< 0.05$ (DMRT).

Table 6 Effect of bromadiolone on erythrocyte enzymatic antioxidant markers

S.No	Erythrocyte enzymatic Antioxidant Markers	Group I Control	Group II 12 hrs.	Group III 24 hrs.	Group IV 48 hrs.	Group V 72 hrs.	Group VI 96 hrs.	Group VII 120 hrs.
1	SOD	115.0±2.0^a	112.1±2.02^b	108.0±2.06^c	98.21±2.04d	83.12±2.08e	62.15±2.12^f	55.01±2.01^g
2	CAT	130.3±1.86^a	125.4±1.81^b	118.6±1.58^c	108.12±1.45^d	85.12±1.32e	68.25±1.22^f	46.34±1.02^g
3	GPx	75.1±2.2^a	74.83±2.01^b	71.23±1.94^c	65.56±1.45^d	40.23±1.12e	34.14±1.09^f	28.76±1.02^g
4	GST	0.45±0.523^a	0.43±0.489^b	0.39±0.468^c	0.34±0.397^d	0.25±0.312e	0.21±0.301^f	0.15±0.287^g
5	GR	0.35±0.383^a	0.32±0.378^b	0.28±0.356^c	0.25±0.312^d	0.18±0.267e	0.15±0.234^f	0.13±0.211^g

Values are given as mean±SD from six rats in each group. $^{a–g}$ Values with different superscript letter (a–g) in the same row differ significantly at $p< 0.05$ (DMRT).

Table 7 Effect of Bromadiolone on erythrocyte non enzymatic antioxidant markers

S.No	Erythrocyte non enzymatic antioxidant markers	Group I Control	Group II 12 hrs.	Group III 24 hrs.	Group IV 48 hrs.	Group V 72 hrs.	Group VI 96 hrs.	Group VII 120 hrs.
1	GSH (umol/g Hb)	5.79±0.70^a	5.72±0.65^b	5.65±0.59^c	4.89±0.52^d	4.31±0.45^e	3.95±0.32^f	3.23±0.21^g
2	VIT.-C (mg/dL)	1.60±0.02^a	1.57±0.04^b	1.55±0.05^c	±1.51±0.08^d	1.48±0.10^e	1.25±0.13^f	1.18±0.16^g
3	VIT.E (mg/dL)	1.25±0.07^a	1.23±0.09^b	1.20±0.11^c	1.17±0.15^d	1.13±0.17^e	1.09±0.19^f	1.02±0.22^g

Values are given as mean±SD from six rats in each group. $^{a–g}$ Values with different superscript letter (a–g) in the same row differ significantly at $p< 0.05$ (DMRT).

Whereas, the rat group 24hrs.fed with BDL showed slight changes in the lipid peroxidation markers when compared to 12hrs.and control rats.

Effect of bromadiolone on enzymatic antioxidants

The enzymatic antioxidant level in the control and experimental rats were depicted in Table 6. Rats fed with BDL for 48hrs, 72hrs, 96hrs and 120 hrs significantly ($P < 0.05$) decreased the level of erythrocytes enzymatic antioxidant when compared with control rats. Similarly a significant($P < 0.05$) decrease in the level of enzymatic antioxidants was also observed in the rats fed with BDL for 24 hrswhen compared with 12 hrs and control rats.

Effect of bromadiolone on non enzymatic antioxidants

Table 7 shows the changes in the levels of erythrocytes nonenzymatic antioxidants, suchas GSH, vitamin C, and vitamin Ein the control and experimental rats. Rats fed with BDL for 48hrs, 72hrs, 96hrs and 120 hrs significantly ($P < 0.05$) decreased the level of erythrocytes non enzymatic antioxidant when compared with control rats. A significant ($P < 0.05$) decrease in the level of non-enzymatic antioxidants were also observed in the rats fed with BDL for 24 hrswhen compared with control and 12 hrs treated rats.

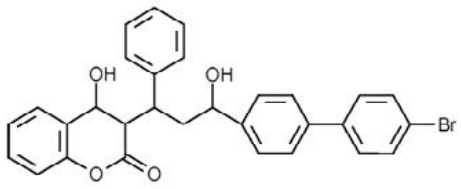

Fig. 1

DISCUSSION

Blood is a body fluid in humans and other animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from the same and other cells. In the present study we intended to evaluate the bromadiolone impact on blood parameters of *B.bengalensis*rats. Bromadiolone (BDL) consumption is associated with the production of free radicals which can react with polyunsaturated fatty acids to yield lipid hydro peroxides which in turn initiates a lipid-radical chain reaction leading to oxidative damage to cell (Anon, 1984). In the present study, the rat fed with bromadiolone significantly decreased Hb, RBCs, RDW, and HCT wasobserved following exposure of bromadiolone in rats. This haematological alteration might be due to effect of BDL on activity of key enzyme of heme synthesis. Moreover BDL also inhibit the conversion of

coproporphyrinogen III to protoporphyrin IX leading to reduction in haemoglobin production and shortened life span of erythrocytes (Revathi and Yoganantha, 2006).

In the present study revealed the impact on the blood erythrocyteindices in response to exposure of BDL in rats. The levels of MCV, MCH and MCHC were significantly decreased in bromadiolone treated rats which are in line with the previous report of (Helal *et al*., 1974). This may be as a result of anemia oronset of glycosylation process because the reactive oxygen speciesgenerated during bromadiolonemetabolism has being implicated in red celldamage (Rao *et al*., 2003).

White blood cells (WBCs), also called leukocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. Leukocytes are found throughout the body, including the blood and lymphatic system (Maton *et al*., 1997). Five different and diverse types of leukocytes exist. These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocyte. In the present exploration rat fed with bromadiolone exhibits a significant decrease in the levels of Lymphocyte, Neutrophils, Monocyte, Eosinophil, Basophils in the blood. The result of the present study in accordance with the previous report (Shamsuddin *et al*., 1981).This might be due to direct toxic action of BDL onleucopoiesis in lymphoid organs. Decrease in total lymphocytes isdirectly related with either their decreased productionfrom the germinal center of lymphoid organs orincreased lysis due to presence of BDL in the body.

Platelets, also called thrombocytes, are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clogging blood vessel injuries.The anticoagulantBDL inhibit the recycling of vitamin K1, a cofactor of primaryimportance for postribosomal carboxylation (activation)of blood clotting factors II, VII, IX and X by the enzyme vitamin K-dependentcarboxylase, maintaining the active form of vitamin K(Petterinoand Paolo 2001; Smith *et al*., 2000). By the enzyme vitamin K-dependentcarboxylase, the active vitamin K is transformed into aninactive epoxide that is thereafter reconverted intovitamin K (vitamin K quinone), by the enzyme vitamin Kepoxide reductase. Anticoagulant rodenticides inhibit vitamin K epoxidereductase, resulting in a lack of active vitamin K and affect the blood clotting. Our result of the present study revealed a significant decreased level of thrombocytes indices in BDL treated rats. This could be due to inactivation of Vit. K by BDL toxicity via inhibition of Vit. K epoxide reductase in the blood of *B.benglensis.*

In the present study a significant increase lipid peroxidation product such as TBARS, LOOH, PC, and CD was observed in BDL treated rat. This might be due to BDL induced free radical, and its content could reflect the increased level of lipid peroxidation products in the blood that will promotes the degradation of RBC membrane integrity and cell viability (Feng *et al*., 2012). This result is good in accordance with the previous report of (valchev *et al*.,2008). The carbonyl derivatives of proteins may result from oxidative modification of amino acid side chains and ROS-mediated peptide cleavage in erythrocytes.

The enzymatic antioxidants are considered to be the first line of cellular defense against toxic stress mediated injury. The measurement of these antioxidant enzymes is an appropriate indirect way to assess the pro-oxidant–antioxidant status in BDL-induced toxicity. SOD and CAT mutually function as important enzymes in the elimination of ROS and reactive nitrogen species. SOD is an enzyme responsible for the conversion of superoxide radicals into less harmful products like hydrogen peroxide, while CAT brings about the reduction of hydrogen peroxide and protects tissues from the highly reactive hydroxyl radicals (Brioukhanov and Netrusov, 2004). GPX is a selenium containing enzyme that uses glutathione in decomposing hydrogen peroxides to nontoxic products. In the present study upon administration of BDL on the activities of SOD, CAT, GPX, GST and GR, in erythrocytes were significantly decreased. This may be due to enhanced level of BDL induced toxicity led to RBC membrane damage through which inhibits the antioxidant status in serum of rats. Rodenticides has been well documented for its anticoagulant and toxicity in *B. bengalensis* for the control in crop fields (Singlaand Kaur, 2015).

Red blood cells are more prone to oxidative damagedue to high content of polyunsaturated fatty acids, iron and oxygen (Heebal, 1986). The present study revealed the alterations in the levels of nonenzymatic antioxidants in blood serum in response to BDL intoxication. Levels of reduced glutathione (GSH), Vitamins C and Vitamins E were significantly decreased with BDL administration at various time intervals. This is may be mainly due to the surplus production of free radicals induced by BDL toxicity led to decrease the availability of non enzymatic antioxidant in blood of *B.bengalensis.*

In conclusion, Present studies consequently divulge that the bromadiolone induced toxicity against *B. bengalensis*blood markers at different time intervals. BDL administration via bait at doses 100mg/kg/BW of different time interval 12 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrsoutcome significantly altered the hematological parameters, oxidative stress markers and antioxidant status in rats. The further studies are required for the molecular action of bromadiolone impact against control of *B.bengalensis*

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