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RESEARCH ARTICLE

The effect of Bromadiolone-Second generation anticoagulant rodenticide on hepatotoxicity in lesser bandicoot rat Bandicota bengalensis (Gray and Hardwicke)

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Abstract

The lesser Bandicoot rat, *Bandicota bengalensis* Gray and Hardwicke are considered as the most important mammalian pests due to the damage they cause to agricultural crops and stored food products. Bromadiolone (BDL), a second generation anticoagulant rodenticide was tested against *B. bengalensis* for its effects on the liver at varying time intervals. Seven groups of six animals each were selected for the experiment. Animals were fed with bromadiolone (0.005%) for 6 days in no-choice test in the form of cake bait (Mixed with wheat, powdered sugar and groundnut oil at a ratio of 96:2:2.) at 12, 24, 48, 72, 96 and 120 hrs. Control animals were maintained for each time interval. Hepatotoxicity of BDL was determined by increased levels of serum hepatospecific markers and total bilirubin, along with increased levels of thiobarbituric acid reactive substances, lipid hydroperoxides, protein carbonyl content, and conjugated Dienes. The hepatotoxic nature of BDL was further evidenced by the decreased activity of enzymatic and nonenzymatic antioxidant levels in liver. BDL treated rats also showed increased DNA damage and histological changes in the hepatocytes. Thus, the results of the present study clearly demonstrate that BDL has strong anticoagulant and rodenticidal properties that induced hepatic injury in *B. bengalensis.*

INTRODUCTION

Rodents are considered as the most destructive mammalian pests due to the damage they cause to standing crops and stored food products (Tobin and Fall, 2006; Santra and Manna, 2008). The lesser Bandicoot rat, *Bandicota bengalensis* Gray and Hardwicke have been reported as one of the major pest in crop fields throughout Southeast Asia (Gogoi and Borah, 2013). The species is also known to spread many diseases to man and livestock (Castillo et al., 2003; single et al., 2008; Meerburg, 2009). Use of rodenticides is the most common method to control rodents. However, excessive use of rodenticides may cause environmental hazards, including non-target toxicity. Rodenticides are broadly classified as first generation and second generation rodenticides. The first generation rodenticides include quick acting acute poisons such as zinc phosphate and barium carbonate. Second generation rodenticides include slow acting multiple dose poisons such as rodafarin and rotafin anticoagulants (Revathi and Yogananda, 2006).

Bromadiolone (BDL) is a second generation anticoagulant rodenticide. The effect of bromadiolone on haematology, histology and DNA damage has not been studied. In this current study analysis of bromadiolone in rodents is studied considering that this chemical would probably enter the food chain. Also, considering this would ultimately affect the physiological mechanism in higher vertebrates (Revathi and Yogananda, 2006). The mechanism of intoxication with anticoagulants is via a specific inhibition of blood coagulation. Vitamin K is needed for the functional synthesis

of coagulation factors II, VII, IX and X. The most common vitamin K-responsive coagulopathy is anticoagulant rodenticide intoxication (Samama et al., 2002; Mount et al., 2003). Blood vessels lose their elasticity, and subsequently ruptures of large blood vessels occur, clinically manifested by massive haemorrhages and hematomas (Radi and Thompson, 2004).

The Liver is one the major target organ for any ingested toxicant in an animal or human beings. This could be due to the major site for the metabolism of all the chemicals (Thangapandiyan et al., 2013). The test species selected for the above experiment are *B. bengalensis*. The present study was intended to evaluate the rodenticidal effect of bromadiolone in the liver of *B. bengalensis* to estimate the pathological, biochemical and molecular effects at varying time intervals viz., 12 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, and 120 hrs.

2. Materials and Methods:

2.1. 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

2.2. Animals and diet:

Male *B. bengalensis* (150-250g weight) were live-trapped from crop fields in and around Sethur Village, Thirunallar Commune, Pondicherry (UT), 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.

2.3. 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 animal 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 7 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 LD50 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 bromadiolon (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.

After the specific period of time interval allotted to each group, one rat was randomly selected and sacrificed under the rats were anaesthetized using cotton wool soaked in chloroform vapours. When they became unconscious, they were quickly brought out of the jar. The liver was weighed and collected used for histopathological examination. The liver tissue was homogenized in 5.0 ml of 0.1M Tris-HCl buffer (pH 7.4) solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

2.4. Liver serum marker enzymes:

The activities of serum AST, ALT, ALP, and LDH and total bilirubin were assayed using commercially available diagnostic kits (Sigma Diagnostics (I) Private, Ltd., Baroda, India). GGT activity was determined following the method of (Rosalki et al., 1970) using γ-glutamyl-*p*-nitroanilide as the substrate. Serum bilirubin was estimated by the method of Malloy and Evelyn (1937), based on the Van den Bergh reaction

2.5. Liver lipid peroxidation markers:

Lipid peroxidation in the liver was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides as described by (Niehaus and Samuelsson, 1968) and (Jiang et al., 1992), respectively. As a hallmark of protein oxidation, total protein carbonyl content was determined in the liver by the spectrophotometric method described by (Levine et al., 1990) and expressed in nanomoles of carbonyl per milligram of protein.

2.6. Estimation of Vit. KO reductase activity:

The vitamin KO reductase activity was measured as described (Thijssen, 1987) with a slight modification: to a reaction mixture of 95 p1 0.02M Tris, 0.15M KCI buffer (pH7.4), containing 0.4 mg of microsomal protein was added 2µl of vitamin KO, 2mgml $^{-1}$ at 1% Triton X-100 in Tris buffer. After a preincubation of 3 min at 30'C the reaction was started by the addition of 5 p1 of dithiothreitol solution, 0.1 Min Tris buffer. The reaction was stopped after 10min incubation by the addition of 1.4 ml of a 0.02 MAgNO2/ isopropanol (5/9, v/v) mixture. Detection was by u.v. monitoring at 313 nm.

2.7. Determination of non enzymatic antioxidants

GSH content in the liver homogenate was determined by the method of (Moron et al., 1979) based on the reaction with Ellman'sreagent (19.8 mg dithionitrobis benzoic acid in 100 ml of 0.1%sodium citrate). 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.

2.8. Assay of enzymatic antioxidants

Superoxide dismutase (SOD) activity was determined following the method of (Kakkar et al., 1984), in which the inhibition of the formation of NADPH-phenazinemetho sulphatenitrobluetetrazolium formation was measured spectrophotometrically at 560 nm. Catalase (CAT) activity was assayed calorimetrically as described by (Sinha, 1972) using dichromate acetic acid reagent. Glutathione peroxidise (GPX) activity was assayed following the method of (Rotruck et al., 1973) based on the reaction between glutathione remaining after the action of GPX and 5, 5=-dithiobis (2-nitrobenzoic acid) to form a complex that absorbs maximally at412 nm. Glutathione S-transferase (GST) activity was determined spectrophotometrically, following the method of (Habiget et al., 1974) using dichloro-2, 4-dinitrobenzene as the substrate. Glutathione reductase (GR), which uses NADPH to convert metabolized glutathione (GSSG) to the reduced form, was assayed by the method of (Horn and Burns, 1978).

2.9. Liver DNA fragmentation assay:

Agarose gel electrophoresis was performed to verify DNA fragmentation (Hebert et al. 1996). The liver tissue was homogenized using 5 ml of lysis buffer (50 mmol·l^{-1}Tris-HCl (pH 8.0), 10 mmol·L^{-1}NaCl, 10 mmol·l^{-1} EDTA, 100 mg·ml^{-1} proteinase K, and 0.5% SDS) and incubated for 1 h at 50 °C. Ten microlitres of100 µg·ml^{-1}ribonuclease A was added to the mixture and incubated for an additional hour at 50 °C. Tissue samples were treated with 1 ml phenol followed by extraction with a chloroform isoamyl alcohol mixture. The aqueous phase was treated with25-50 L of 3 mol·L^{-1} sodium acetate (pH 5.2) and one volume of ethanol, shaken gently, and left at −20 °C overnight. The precipitate was collected by centrifugation at 12 000 x*g* for 20 min. The pellet was rinsed with 1 ml of 70% ethanol and spun for 10 min. The supernatant was discarded and the pellet was air dried at room temperature and later dissolved in 0.5-1.0 ml of double distilled water. DNA was precipitated in cold ethanol at −20 °C and finally dissolved in 0.5 ml of buffer. The DNA sample was loaded in1.0% agarose gel containing 0.5 µg·ml−1 ethidium bromide, electrophoresed at 80 V, and visualized under a UV transilluminator.

3.0. Histopathology of liver

For qualitative analysis of liver histology, the tissue samples were fixed for 48 h in 10% formalin-saline and dehydrated by passing successfully through different mixtures of ethyl alcohol and water, cleaned in xylene, and embedded in paraffin. Sections of the tissue (5-6 µm thick) were prepared using a rotary microtome, stained with haematoxylin and eosin dye and then mounted in a neutral deparaffinized xylene medium for microscopic examinations.

3.1. Statistical analysis

Data are presented as the mean ± SD and were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS version15.0 (SPSS Inc., Cary, North Carolina, USA) and the individual comparisons were obtained by Duncan's multiple range test. Values for $P < 0.05$ were considered statistically significant.

4. Results:

4.1. Effect of bromadiolone on liver serum marker enzymes:

The level of serum liver marker enzymes and bilirubin in control and experimental rats was presented in Table 1. BDL treated rats since 48 hrs, 72 hrs, 96 hrs and 120 hrs showed a significant (P<0.05) increase levels of liver serum marker enzymes (AST, ALT, ALP, LDH, and bilirubin) when compared with control rats. Whereas

group 2, and 3 treated with BDL shows moderate changes occurred in the serum of liver marker enzyme when compared with control rats.

4.2. Effect of bromadiolone on lipid peroxidation:

Table 2 shows the changes in the levels of lipid peroxidation products in the control and experimental animals. The rats fed with BDL for 48 hrs, 72 hrs, 96 hrs and 120 hrs, showed a significant increase *(P<0.05)* levels of TBARS, LOOH, PCC and CD when compared with control rats. Whereas, the rat group 24 hrs. fed with BDL showed slight changes in the lipid peroxidation markers when compared to 12 hrs. and control rats.

4.3. Effect of Bromadiolone on Vit.KO reductase activity:

Figure 2 shows the effect of bromadiolone on Vit. KO reductase activity in control and experimental rats. There was a significant (P<0.05) decrease level of Vit. KO reductase activity in rat fed with BDL for 120hrs followed by 96 hrs, 72 hrs, 48 hrs. and 24 hrs. in experimental rats when compared with control rats. Whereas rat fed with BDL for 12 hrs. showed slight changes when compared with control rats.

4.4. Effect of bromadiolone on non enzymatic antioxidants:

Table 3 shows the changes in the levels of liver non enzymatic antioxidants, namely, GSH, TSH, vitamin C, and vitamin E in the liver of control and experimental rats. Rats fed with BDL for 48 hrs, 72 hrs, 96 hrs and 120 hrs. significantly ($P < 0.05$) decreased the level of liver 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 hrs when compared with control and 12 hrs treated rats.

4.5. Effect of bromadiolone on enzymatic antioxidants:

The enzymatic antioxidant level in the control and experimental rats were depicted in Table 4. Rats fed with BDL for 48 hrs, 72 hrs, 96 hrs and 120 hrs. significantly ($P < 0.05$) decreased the level of liver 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 hrs. when compared with control and 12 hrs. treated rats.

4.6. Effect of bromadiolone on liver DNA fragmentation

Figure 3 shows the level of DNA damage in the liver of control and experimental rats. Rat groups were fed with BDL for 48 hrs (Lane 4), 72 hrs (Lane 5), 96 hrs (Lane 6), and 120 hrs (Lane 7) show a significant (P<0.05) increase DNA damage when compared with control rats (Lane 1). Whereas, rat fed with BDL for 12 hrs (Lane 2) and 24 hrs (Lane 3) showed no DNA damages when compared with control rats.

4.7. Effect of bromadiolone on histopathology

Figure 4 shows a representative photomicrograph of an intact rat liver from the control group (Fig. 4A). The histoarchitecture pattern of liver was almost normal in rats fed with BDL 12 hrs and 24 hrs (Fig. 4B&4C). The liver cells of animals exposed for 48 hrs (Fig. 4D), 72 hrs (Fig. 4E), 98 hrs (Fig. 4F) and 120 hrs (Fig. 4G) showed several histological changes, such as extensive degeneration of hepatocytes with focal necrosis, bridging necrosis, inflammation, vacuolization, inflammatory cell infiltration, portal inflammation and fatty degenerative changes.

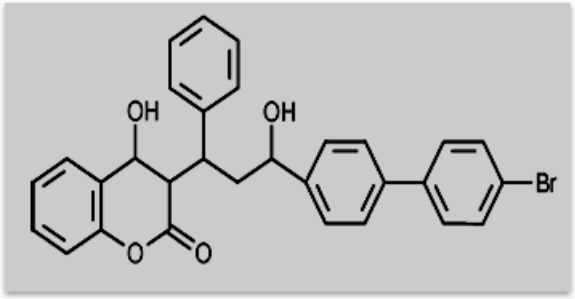

Fig. 1

Fig.1. Chemical structure of Bromadiolone

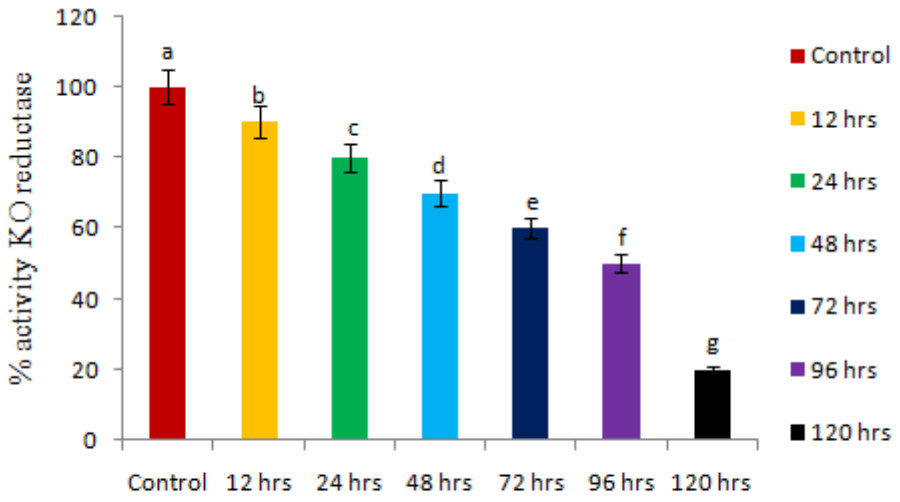

Fig. 2

Fig.2. Effect of bromadiolone on Vit.KO reductase activity in control and experimental rat groups (12 hrs-120 hrs). Values are given as mean ± SD for six rats in each group. Values with different superscript letter (a-f) differ significantly at P<0.05 (DMRT).

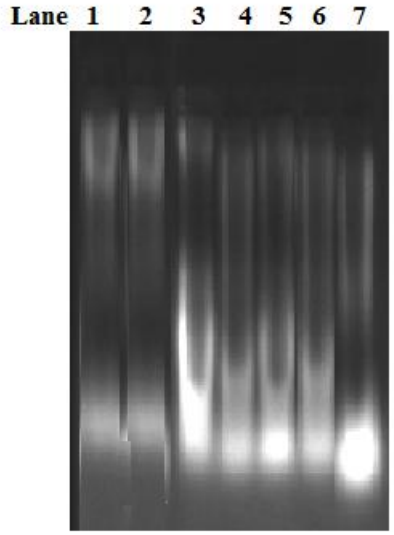

Fig. 3

Fig.3. Effect of bromadiolone on liver DNA damage in control and experimental rats. Control rats (Lane 1) and rat fed with BDL for 12 hrs (Lane 2) shows no DNA damage. Rat fed with BDL for 24 hrs (Lane 3) showed more DNA damage followed by 48 hrs (Lane 4), 72 hrs (Lane 5), 96 hrs (Lane 6) and 120 hrs (Lane 7) in rats.

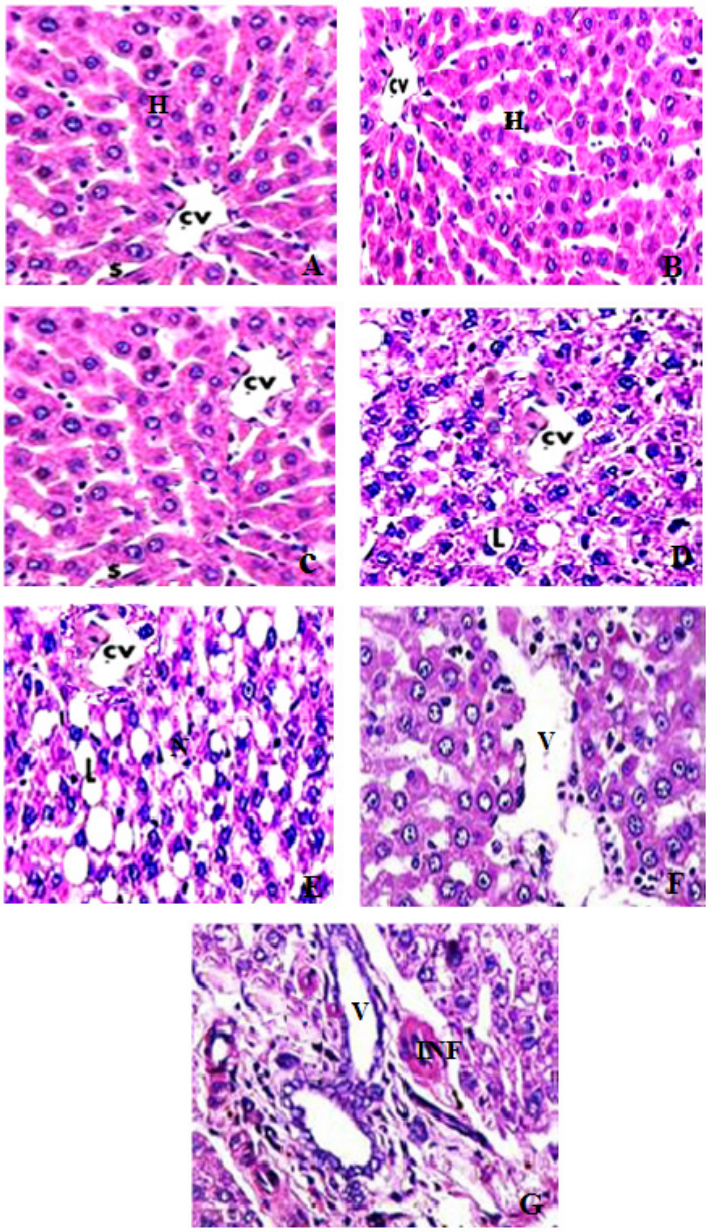

Fig. 4

Fig.4. Representative photomicrographs of liver sections of control and experimental rats stained with haematoxylin and eosin. (A) Section of control liver showing normal arrangement of hepatocytes (H) with central vein (CV) (B) Section of liver fed with BDL for 12 hrs. showing the normal histoarchitecture pattern (C) Section of liver fed with BDL for 24 hrs. showing minimal hepatic damages (D) Section of liver treated with BDL for 48 hrs. showing severe necrotic changes, fatty degeneration of hepatocytes, and complete (E) Section of liver fed with BDL for 72 hrs. showing derangement of hepatic cords and vacuolar spaces (V). (F&G) Section of liver fed with BDL for 98 hrs and 120 hrs. showing degeneration of hepatocytes with necrosis (N), vacuolization, and inflammatory cell infiltration (INF). Magnification ×40.

Table 1. Effect of bromadiolone in the activities of liver serum marker enzymes aspartate transaminase (AST), alanine transaminase (ALT), alkaline ph osphatase (ALP) and lactatedhydrogenase (LDH) in the bromadiolone treated control and experimental rats.

Groups	Control	Group 12 hrs	Group 24 hrs	Group 48 hrs	Group 72 hrs	Group 96 hrs	Group 120 hrs
AST (IU/L)	63.89 ± 5.34[a]	64.78 ± 3.72[a]	69.59 ± 4.63 [ab]	76.39 ± 4.75[c]	94.45 ± 4.96[d]	103.24 ± 5.23[e]	132.20 ± 6.23[f]
ALT (IU/L)	27.89 ± 2.87[a]	29.24 ± 2.79[a]	39.36 ± 2.92 [ab]	47.79 ± 3.13 [c]	51.85 ± 3.57 [d]	62.38 ± 3.68[e]	69.13 ± 3.70[f]
ALP (IU/L)	74.61 ± 5.43[a]	77.59 ± 4.12[a]	83.57 ± 5.78 [ab]	93.83 ± 5.83 [c]	102.41 ± 5.97 [d]	108.58 ± 6.73[e]	122.92 ± 8.13[f]
LDH (IU/L)	109.82 ± 8.81[a]	112.36 ± 9.85[a]	135.85 ± 10.66 [ab]	147.45 ± 11.84[c]	159.15 ± 12.17[d]	169.31 ± 12.61[e]	176.24 ± 13.41[f]
Bilirubin (mg/dl)	0.45 ± 0.02[a]	0.48 ± 0.03[a]	0.54 ± 0.04 [ab]	0.66 ± 0.05 [c]	0.79 ± 0.06 [d]	0.88 ± 0.07 [e]	0.97 ± 0.08[f]

Values are given as mean ± S.D. from six rats in each group. Values not sharing a common superscript letter (a-f) differ significantly at p<0.05 (DMRT).

Table 2: Changes in the levels of hepatic lipid peroxidation (TBARS) Lipid hydroperoxides (LOOH) protein carbonyl (PC) content and conjugated dienes (CD) in the bromadiolone treated control and experimental rats.

Parameters	Control	**Group 12 hrs**	**Group 24 hrs**	**Group 48 hrs**	**Group 72 hrs**	**Group 96 hrs**	**Group 120 hrs**
TBARS	8.17 ± 0.42[a]	8.23±0.47[a]	9.35±0.65[b]	9.89±0.97[c]	11.46±1.12[d]	12.23±1.78[e]	13.12±1.87[f]
LOOH	0.82 ± 0.56[a]	0.85±0.57[a]	0.96±0.62[b]	0.99±0.69[c]	0.108±0.83[d]	0.119±0.92[e]	0.124±0.98[f]
CD	68.38±4.69[a]	69.39±4.71[a]	74.76±4.98[b]	79.23±5.11[c]	88.11±5.35[d]	92.13±5.89[e]	97.25±5.93[f]
PC	3.37 ± 0.05[a]	3.45±0.10[a]	3.78±0.18[b]	4.12±0.27[c]	4.89±0.39[d]	5.09±0.49[e]	5.65±0.56[f]

Values are mean ± SD for 6 rats in each group; [a-f] Values are not sharing a common superscript letter (a-f) differ significantly at *p*<0.05 (DMRT).

Levels of TBARS were expressed as nmol/100g wet tissue.
Levels of LOOH and CD were expressed as nmol/100g wet tissue.
Levels of PC were expressed as nmol/mg protein.

Table 3. Changes in the levels of vitamin - C, vitamin - E, reduced glutathione (GSH) and total sulfhydryl groups (TSH) in the bromadiolone treated liver of control and experimental rats.

Parameters	Control	**Group 12 hrs**	**Group 24 hrs**	**Group 48 hrs**	**Group 72 hrs**	**Group 96 hrs**	**Group 120 hrs**
Vitamin - C (µM/mg)	2.95 ± 0. 78[a]	2.92±0.75[b]	2.75±0.68[b]	2.49±0.61[c]	2.11±0.51[d]	1.89±0.38[e]	1.65±0.21[f]
Vitamin - E (µM/mg)	2.36 ± 0.49[a]	2.32±0.46[a]	2.21±0.39[b]	2.01±0.31[c]	1.98±0.21[d]	1.87±0.17[e]	1.65±0.11[f]
GSH (µg/mg)	19.54 ± 1.26[a]	19.51±1.22[a]	19.46±1.17[b]	19.11±1.11[c]	18.78±1.06[d]	18.65±1.03[e]	17.06±0.92[f]
TSH (µg/mg)	16.11± 1.23[a]	16.07±1.21[a]	16.16±1.17[b]	15.87±1.09[c]	15.23±0.981[d]	15.07±0.891[e]	14.67±0.801[f]

Values are given as mean ± S.D. from six rats in each group. Values not sharing a common letter (a-f) differ significantly at p<0.05 (DMRT).

Table 4. Changes in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and glutathione reductase (GR) in bromadiolone treated liver of control and experimental rats

Groups	Control	**Group 12 hrs**	**Group 24 hrs**	**Group 48 hrs**	**Group 72 hrs**	**Group 96 hrs**	**Group 120 hrs**
SOD	9.89 ± 0.585^{a}	9.86 ± 0.581^{a}	9.76 ± 0.576^{b}	9.34 ± 0.521^{c}	8.83 ± 0.487^{d}	8.23 ± 0.455^{e}	7.78 ± 0.401^{f}
CAT	94.25 ± 4.85^{a}	92.22 ± 4.79^{a}	89.79 ± 4.72^{b}	81.71 ± 4.67^{c}	75.87 ± 4.55^{d}	71.76 ± 4.45^{e}	68.91 ± 4.12^{f}
GPx	9.86 ± 0.534^{a}	9.83 ± 0.532^{a}	9.81 ± 0.529^{b}	9.74 ± 0.524^{c}	9.23 ± 0.518^{d}	9.17 ± 0.509^{e}	9.01 ± 4.94^{f}
GST	9.75 ± 0.47^{a}	9.72 ± 0.45^{a}	9.69 ± 0.42^{b}	9.55 ± 0.38^{c}	9.23 ± 0.29^{d}	9.18 ± 0.23^{e}	9.11 ± 0.21^{f}
GR	0.64 ± 0.35^{a}	0.62 ± 0.32^{a}	0.59 ± 0.29^{b}	0.53 ± 0.25^{c}	0.48 ± 0.21^{d}	0.42 ± 0.19^{e}	0.38 ± 0.13^{f}

Values are given as mean ± S.D. from six rats in each group. Values not sharing a common superscript letter (a-f) differ significantly at P<0.05 (DMRT).
SOD –one unit of enzyme activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute/mg protein; CAT–µmol of H_2O_2 utilized/min/mg protein; GPX–µmol of GSH consumed/min/mg protein; GST–µmol of CDNB-GSH conjugate formed/min/mg protein; GR - µmol of NADPH oxidized/min/mg protein.

5. Discussion

B. bengalensis is the most important species. Widespread in distribution, it causes serious economic losses to grow crops such as rice, wheat, sugar cane, and groundnut (Khan et al., 1981). In the present study, biochemical and histomorphological changes induced by bromadiolone treatment in male lesser Bandicoot rat were studied.

The increase in various biochemical parameters showed the effect of Bromadiolone treatment on metabolism of animals and this effect is closely related to the dose level, as an increase in the various biochemical parameters is in a dose dependent manner. Increased levels of AST, ALT, ALP, LDH and bilirubin are usually indicative of liver damage in animals and humans (Selvakumar, et al., 2013). ALP considered as important integrity markers of cellular damage and is secreted by lysozymes. A significant increase in the levels AST, ALT, ALP, LDH and bilirubin were also found in Bromadiolone treated rats which are in line with the previous report of (Parul Dhar and Neena Singla, 2014). Hence, change in activity of these enzymes is generally related to intensity of cellular damage (Sangha, et al., 2013). Abnormalities in serum marker enzymes are not indicative of a specific disease but of a condition that alters the tissue responsible for the balance between protein synthesis and catabolism or mechanical loss.

In the present study, increased in lipid peroxidation markers was observed in BDL treated rats, which might be due to increased cell membrane permeability or cell membrane damage of hepatocytes caused by bromadiolone toxicity. These findings are in accordance with (Maroni, et al., 2000). Amongst all treatment groups increase TBARS, LOOH, PC, and CD was highest in 120 hrs, followed by 98 hrs, 72 hrs and 48 hrs. as compared to control group. The increase in lipid peroxidation might be due to the damage of the liver resulting in liberation of these marker enzymes results of ROS (Thangapandiyan and Miltonprabu, 2013). Increase lipid peroxidation level observed in the present study is in agreement with the findings of (Maroni et al, 2000). The increased lipid peroxidation might be due to abundance ROS accumulation in the liver by bromadiolone toxicity.

Vitamin K is a necessary cofactor in activating clotting factors II, VII, IX, and X by carboxylation. Without vitamin K, these coagulation proteins will remain in a non-functional, a precursor state (Ivan valchev et al., 2008). In the present investigation, BDL is rodenticides interfere with Vit. KO reductase and significantly decreased levels in experiments, rats when compared with control rats. This result of the present study in accordance with the previous report of (Mosterd and Thijssen., 1991). Vitamin K is consumed by carboxylation of the proteins and is present as vitamin K epoxide, which cannot activate clotting proteins (Mount, 1988). Normally, the body converts vitamin K epoxide back to active vitamin K via the enzyme vitamin K epoxide reductase. Anticoagulant rodenticides inhibit

this enzyme, resulting in a lack of active vitamin K (Mount, 2004) as a result, concentrations of the clotting factors decrease, since no more precursor protein can be converted to an active form.

Antioxidant defence system mainly involves in the scavenging reactive oxygen species (ROS) to prevent the animal death through organ dysfunctions (Thangapandiyan and Miltonprabu, 2013). Amongst the different antioxidant enzymes, SOD and CAT equally function as essential enzymes in the diminution of ROS. Liver SOD activity in bromadiolone treated rats may be due to the over- production of super oxide radical anions. In order to remove excess free radicals from the system, GST and GPx make use of GSH during their course of reactions. Decrease in GSH, TSH, and vitamin content due to BDL toxicity simultaneously decreased the activities of GST as well as GPx with a simultaneous decline in the activity of GSH regenerating enzyme, GR. This may be due to the blocking of Vit. K epoxide cycles in the liver by bromadiolone in which increase the haemorrhage (William, 1961). In the present study, bromadiolone treated rats showed decreased levels of these antioxidants (non-enzymatic and enzymatic) levels in the liver due to over accumulation of BDL induced ROS toxicity. The rat fed with bromadiolone for 120 hrs. Shows decreased levels of non-enzymatic and enzymatic antioxidant followed by the 96 hrs, 72 hrs, and 48 hrs. mainly due to the blocking of Vit. K activity. This is the first report on bromadiolone treated antioxidant status in *B.bengalensis.*

The DNA fragmentation forms a typical ladder pattern of multiple sized nucleosomal nucleotides, an indicator of DNA destruction through apoptosis in hepatocytes (Miltonprabu and Thangapandiyan, 2015). BDL has potent anticoagulant and biochemically very active, and thus affect DNA because of its strong inhibitory activity with Vit. K by the interaction with hepatocytes (Ivan valchev et al., 2008). In the present exploration, BDL treated rat's showed significant DNA damage in 120 hrs. Treated rats followed by 96 hrs, 72 hrs and 48 hrs. This might be due to inhibit the recycling of vitamin K1, a cofactor of primary importance for post ribosomal carboxylation (activation) of blood clotting factors II (prothrombin), VII (proconvertin), IX (Christmas factor), and X (Stuart-Prower factor), by the enzyme vitamin K-dependent carboxylase, lead to hepatic DNA damage in rats.

The histological result also strongly supports our biochemical findings that, BDL treated rats showed significant damage in the histology of experimental rat liver when compared with control rats. This study shows the altered hepatic histoarchitecture such as necrosis, inflammatory cell infiltration, vacuolization, dilation of sinusoidal spaces, and inflammation in BDL treated rats. There was a severe damage occurred at 120 hrs. treated rats followed by 96 hrs, 72 hrs and 48 hrs. treated rats. This is mainly due to the various toxic factors (ROS, lipid peroxidation etc.,) of BDL in the liver tissues led to damage normal histoarchitecture in rats.

In conclusion, the intoxications with Bromadiolone are anticoagulant rodenticides in animals are relatively frequent. To date, these preparations have been the only means of effective control of rodent populations, especially *B.bengalensis* in economic crop fields. Therefore, the principles of treatment and observation with regard to the protection of valuable money crops against *B. bengalnsis.*

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Conflict of interest:

None.

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