RESEARCH ARTICLE

Received on: 06/09/2015
Accepted on: 30/09/2015
Published on: 10/10/2015

Corresponding Author
J. Baskaran ;
Department of zoology, Annamalai University, Chidambaram, Tamilnadu, India
Email: jbaskaran.amu@gmail.com

QR Code for Mobile users

Conflict of Interest: None Declared !

Bromadiolone-Second generation anticoagulant rodenticide induced nephrotoxicity on lesser bandicoot rat *Bandicota bengalensis* (Gray and Hardwicke)

N. Sridhar[1], J. Baskaran[1*], S. Thangapandiyan[1], S. Dhanasekaran[1], D. Vasantharaja[2], S. Mahesh babu[1]
[1]Department of zoology, Annamalai University, Chidambaram, Tamilnadu, India.
[2]Department of Zoology, KMCPGS (Pondicherry University), Lawspet, Puducherry, India.

ABSTRACT

The lesser bandicoot rat, *Bandicota bengalensis* Gray and Hardwicke is the most predominant rodent pest species in agricultural and commensal situations. Use of rodenticides is the most common method to control rodents. Bromadiolone (BDL), a second generation anticoagulant rodenticide was tested on *B. bengalensis* for its effects on kidney at varied time intervals. Seven groups of six animals each were selected for experiment. 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 12 hrs, 24 hrs, 48 hrs, 72 hrs 96 hrs and 120 hrs. Results revealed significant alteration in the serum and urinary biomarkers with increased levels of thiobarbituric acid reactive substances, lipid hydroperoxides, protein carbonyl content, and conjugated dienes in BDL treated rats. The toxicity of BDL in kidney was further evidenced by the decreased activity of enzymatic and non-enzymatic antioxidant levels and increased DNA damage and histological changes in the kidney tissues. Thus, the results of the present study clearly demonstrate that BDL has strong rodenticidal and anticoagulant properties that damage the kidney in *B. bengalensis*.

Keywords: Bromadiolone, *Bandicota bengalensis*, Kidney, Nephrotoxicity.

Introduction

Rodents are major pests in many agricultural systems, where they cause significant losses in grain production and damage to stored foods. They are involved in the epidemiology of several diseases [1-2]. *B. bengalensis* may significantly affect crop production and livelihoods of farmers in both developed and developing countries [3]. They cause economic problems because of the damage they inflict on agricultural systems. Damages caused by them lead to huge amount of crop losses and food shortages. In India alone there is $5 billion nearly 25%-30% of stored food grains were damaged by rodents [4]. Rodent pests are responsible for considerable economic losses to food production on a world wide scale [5]. Some of them are carriers of serious diseases like Plague, Leptospirosis, murine typhus, etc [6]. Rodent control with rodenticides is the most popular and effective means of rat control. For the last decades, anticoagulant rodenticides have been widely used management tool to protect crops and stored product against rodents [7-8]. Although the availability of alternative methods of pest control, the use of chemicals plays an important role in integrated pest management. In modern times, rodenticides have been an important element in controling rodent pests [9-10].

Bromadiolone is second generation anticoagulant rodenticide which is commercially available for the control of agricultural pest. It has been used commonly as 0.005% rat bait in the form of cereal-based baits, pastes, tracking powders or wax blocks [11]. The exact mechanism of bromodilone is not known, but directly or indirectly generate the free radicals in which affect the different organs in the body [12]. The one of the important mechanism in which bromadiolone damge the organ is via a specific inhibition of blood coagulation [12] . 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 [13-14]. Blood vessels lose their elasticity, and subsequently ruptures of large blood vessels occur, clinically manifested by massive haemorrhages and haematomes [15-16] . Kidney is an important excretory organ of rat and human. The most important function of the kidney is to maintain a normal physiological condition of the body. The chemical poison present in bromadiolone decreases the excretory capacity of the kidney, by damage via its anticoagulation properties [11-17] . In the available literature there are no complete biochemical, histological and molecular data for kidney

intoxicated with bromadiolone in rats. Thus the reason motivated us to carried out to evaluate the nephrotoxic effect of bromadiolone at varied time intervals viz., 12 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, and 120 hrs in the kidney tissue of *B.bengalensis.*

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 "Moosh Moosh". Bromadiolone wax cake is directly palatable to the test animal. All other chemicals and solvents were of certified analytical grade and purchased from, Himedia Laboratories Pvt. Ltd., Mumbai, or Pondicherry, India. Reagent kits were obtained from National scientific supplier, Pondicherry, India.

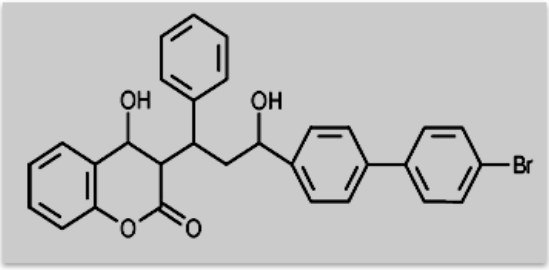

Fig. 1

Fig. 1. Chemical structure of Bromadiolone

2.2. Animals and diet:

Male *B. bengalensis* (150-250g weight) were live-trapped from crop fields in and around Sethur Villege, Thirunallar commune, Karaikal region, Pondicherry (UT), India. In the laboratory, rats were acclimatized individually in cages (36 × 23 × 23) with food and water provided for 6 days before the commencement of the experiment. The rats were kept in a room with 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 in to 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 "weighting balance" after an allotted hour interval, and the quantity of the bait consumed was recorded. The LD_{50} value of bromadiolone is 1.125 mg/kg of 100gm of BDL (0.005%) [18].

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 24 hrs.

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 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 kidney was weighed and collected used for histopathological examination. The kidney 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. Biochemical assays

2.4.1. Estimation of urea, uric acid, creatinine and creatinine clearances

The levels of urea, uric acid and Creatinine in serum and urine were estimated spectrophotometrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt. Ltd., Baroda, India). Creatinine clearance as an index of glomerular filtration rate was calculated from serum Creatinine and 24-h urine sample Creatinine level.

2.4.2. Determination of lipid peroxidation markers in kidney:

Lipid peroxidation in the kidney was estimated calorimetrically by measuring Thiobarbituric acid reactive substances (TBARS) and hydroperoxides as described by [19] and [20], respectively. As a hallmark of protein oxidation, total protein carbonyl content was determined in the kidney by the spectrophotometric method described by [21] and expressed in nanomoles of carbonyl per milligram of protein.

2.4.3. Determination of non-enzymatic antioxidant concentrations:

Reduced GSH was determined by the method of [22] based on the reaction with Ellman's reagent (19.8 mg dithionitrobisbenzoic acid in 100 mL of 0.1% sodium citrate). Total sulfhydryl groups (TSH) in the kidney homogenate were measured after the reaction with dithionitrobisbenzoic acid using the method of [23]. Ascorbic acid vitamin C and vitamin E concentrations were measured by the methods of [24] and [25] respectively.

2.4.4. Determination of activities of antioxidant:

Superoxide dismutase (SOD) activity was determined by the method of [26] in which inhibition of the formation of NADH-phenazinemethosulfate nitrobluetetrazolium formation was measured spectrophotometrically at 560 nm. Catalase (CAT) activity was assayed calorimetrically as described by [27] using dichromate acetic acid reagent. Glutathione peroxidase (GPX) activity was assayed by the method based on the reaction between GSH remaining after the action of GPx and 5,5-dithiobis-2-nitrobenzoic acid to form a complex that absorbs maximally at 412 nm, as described by [28]. Glutathione S-transferase (GST) activity was determined spectrophotometrically by using dichloro-2,4-dinitrobenzene as the substrate, as described by [29]. Glutathione reductase (GR), which uses NADPH to convert glutathione disulfide (GSSG) to the reduced form, was assayed by the method of [30].

2.4.5. Kidney DNA fragmentation assay:

Agarose gel electrophoresis was performed to verify DNA fragmenta tion [31] The kidney 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 of 100 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 with 25–50 L of 3 mol·L^{-1} sodium acetate (pH 5.2) and one volume of ethanol, shaken gently, and left at −20 °C over night. 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. 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

2.4.6. Histopathology of Kidney

For qualitative analysis of kidney 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.

2.4.7. Statistical analysis

Data are presented as the mean ± SD and were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS version 15.0 (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.

3.Result:

3.1. Effect of bromadiolone on serum and urinary nephrotoxic markers

Fig. 2 shows the level of serum and urinary nephrotoxic markers in control and bromadiolone treated rats. There was a significant ($P<0.05$) increase in the levels of serum urea (A), uric acid (B) and creatinine (C) along with decreased levels of creatinine clearance (D) observed at 12 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, and 120 hrs in BDL treated rat serum as compared to that of control. Similarly, Fig. 3 shows a significant ($P< 0.05$) decrease levels of urinary creatinine (A), urea (B) and uric acid (C) were observed at 12 hrs, 24 hrs, 48 hrs, 72 hrs, 96 hrs, and 120 hrs in BDL intoxicated rats when compared to control rats.

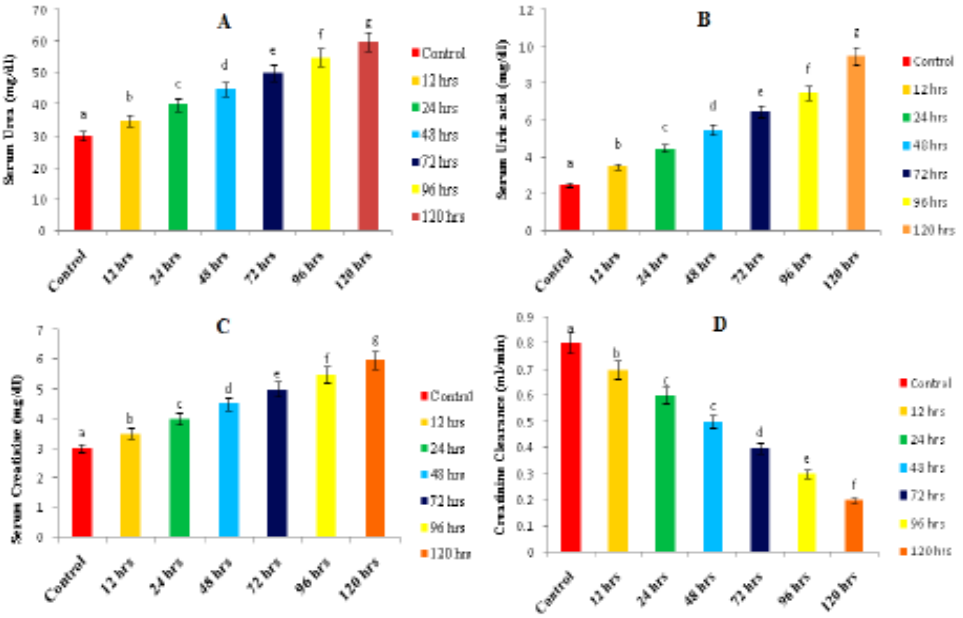

Fig. 2

Fig. 2. Effect of BDL on kidney serum biomarkers in control and experimental rats. A significant increase levels of serum renal merkers such as urea in serum (mg/dL) (Fig. A), uric acid in serum (mg/dL) (Fig. B), creatinine in serum (mg/dL) (Fig. C), and decreased level of creatinine clearance in serum (mL/min) (Fig. D) were observed in BDL treated rats. Statistical significance was determined by one way ANOVA. Bars with different superscript letters (a–g) differ significantly at $P < 0.05$ (DMRT).

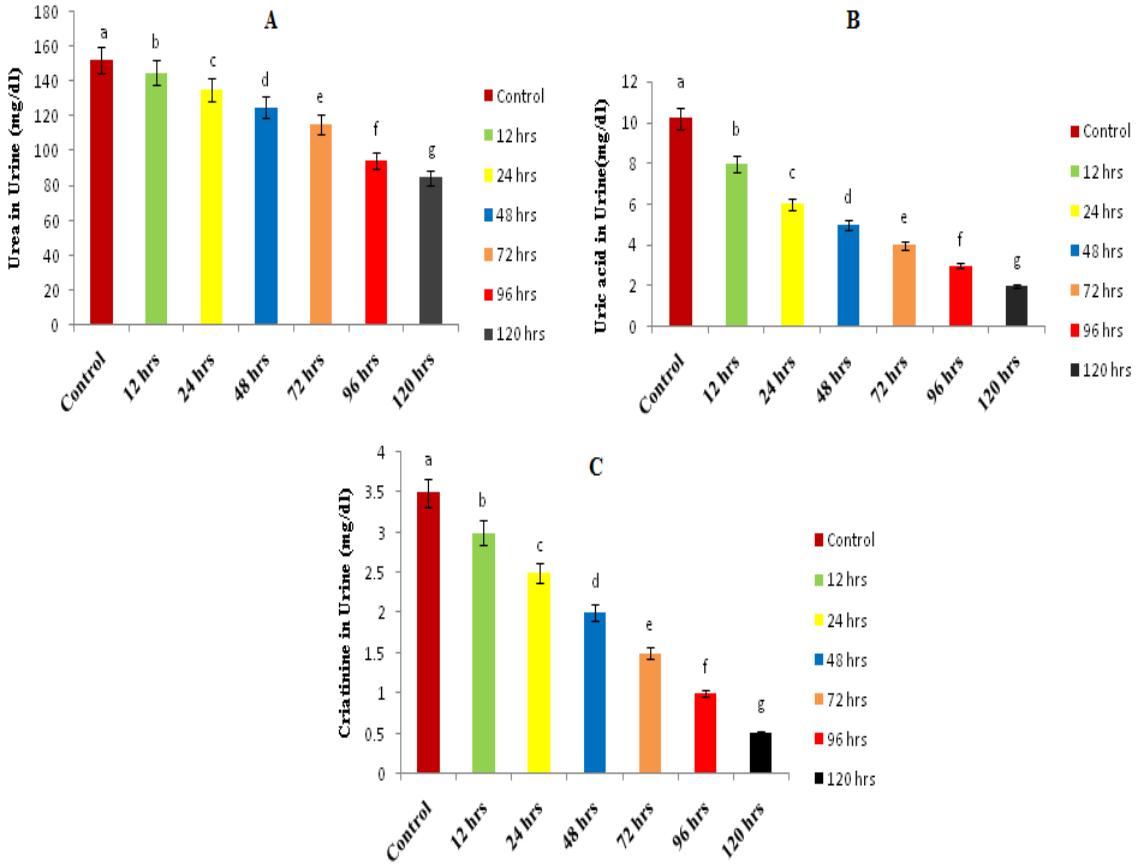

Fig. 3

Fig. 3. Effect of bromadiolone on kidney urinary biomarkers in control and experimental rats.
A significant decrease levels of urinary biomarkers such as urea in urin (mg/dL) (Fig. A), uric acid in urin (mg/dL) (Fig. B), creatinine in urin (mg/dL) (Fig. C) were observed in BDL treated rats when compared to the control rats. Statistical significance was determined by oneway ANOVA. Bars with different superscript letters (a–g) differ significantly at $P < 0.05$ (DMRT).

3.1. Effect of bromadiolone on lipid peroxidation

Table 1 shows the changes in the levels of lipid peroxidation products in control and experimental animals. The rats fed with BDL for 24 hrs, 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 12 hrs fed with BDL showed slight changes in the lipid peroxidation markers when compared with control rats.

Table 1: Changes in the levels of renal lipid peroxidation markers such as TBARS, LOOH, PC, CD in 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	3.17 ± 0.42[a]	3.23±0.47[a]	3.55±0.65[b]	4.89±0.97c	5.46±1.12[d]	6.23±1.78[e]	7.12±1.97[f]
LOOH	0.65 ± 0.06[a]	0.75±0.07[a]	0.82±0.08[b]	0.89±0.09[c]	0.95±0.11[d]	0.109±0.12[e]	0.114±0.14[f]
PC	2.37 ± 0.19[a]	2.45±0.21[a]	2.98±0.28[b]	3.12±0.32[c]	4.34±0.41[d]	5.45±0.55[e]	6.15±0.61[f]
CD	1.14± 0.04[a]	1.16± 0.10[a]	1.20 ±0.12[b]	1.25 ± 0.17[c]	1.30 ±0.20[d]	2.21 ±0.21[e]	2.30± 0.25[f]

Values are given as mean ± SD from six rats in each group. Values with different superscript letters (a–f) in the same column differ significantly at P < 0.05 (DMRT).

3.2. Effect of bromadiolone on non enzymatic antioxidants:

Table 2 shows the changes in the levels of kidney non enzymatic antioxidants, namely, GSH, TSH, vitamin C, and vitamin E in the kidney 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 kidney non enzymatic antioxidant when compared with control rats. A significant (P < 0.05) decrease in the level of non enzymatic antioxidants was also observed in the rats fed with BDL for 24 hrs when compared with control and 12 hrs treated rats.

Table 2: Changes in the levels of vitamin - C, vitamin - E, reduced glutathione (GSH) and total sulfhydryl groups (TSH) in the renal 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**
GSH (µg/mg)	3.55±0.43[a]	3.51±0.41a	3.34±0.39[b]	3.13±0.35[c]	2.01±0.29[d]	1.98±0.22[e]	1.55±0.12[f]
TSH (µg/mg)	11.87±0.73[a]	11.85±0.71[a]	10.55±0.67[b]	10.12±0.61[c]	9.45±0.55[d]	8.12±0.47[e]	7.34±0.32[f]
Vitamin - C (µM/mg)	0.91±0.07[a]	0.89±0.07[a]	0.85±0.06[b]	0.78±0.05[c]	0.71±0.04[d]	0.64±0.03[e]	0.54. ±0.02[f]
Vitamin - E (µM/mg)	0.76 ± 0. 05[a]	0.73±0.05a	0.69±0.4[b]	0.61±0.4[c]	0.55±0.03[d]	0.51±0.02[e]	0.45±0.01[f]

Values are given as mean ± SD from six rats in each group. Values with different superscript letters (a–f) in the same column differ significantly at P < 0.05 (DMRT).

3.3. Effect of bromadiolone on enzymatic antioxidants:

The enzymatic antioxidants level in the control and experimental rats were depicted in Table 3. Rats fed with BDL for 48 hrs, 72 hrs, 96 hrs and 120 hrs significantly (P < 0.05) decreased the level of kidney 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.

Table 3: Changes in the levels of renal enzymatic antioxidants SOD, CAT, GPx, GST, and GR in control and experimental rats.

Parameters	Control	**Group 12 hrs**	**Group 24 hrs**	**Group 48 hrs**	**Group 72 hrs**	**Group 96 hrs**	**Group 120 hrs**
SOD	13.9±0.553[a]	13.1±0.532[a]	11.23±0.521[b]	9.78±0.512[c]	7.83±0.487[d]	5.23±0.455[e]	3.78±0.401f
CAT	46.25 ± 3.45[a]	45.21±3.41[a]	41.89±3.23[b]	37.55±3.01c	32.12±2.89[d]	27.32±2.12[e]	21.67±2.01[f]
GPx	6.55 ± 0.434[a]	6.51±0.431[a]	6.45±0.424[b]	5.67±0.401[c]	5.01±0.367[d]	4.89±0.324[e]	4.21±0.312[f]
GST	6.23±0.32[a]	6.21±0.31[a]	6.15±0.26[b]	5.59±0.23[c]	5.32±0.18d	4.89±0.13[e]	4.54±0.11f
GR	0.54 ± 0.04[a]	0.51±0.04a	0.47±0.03[b]	0.43±0.02c	0.38±0.01d	0.32±0.01[e]	0.38±0.01[f]

Values are given as mean ± SD from six rats in each group. Values with different superscript letters (a–f) in the same column differ significantly at P < 0.05(DMRT). SOD – one unit of enzyme activity was defined as the enzyme reaction that gave 50% inhibition of NBT reduction per minute/mg protein. CAT–mol of H_2O_2consumed/min/mg protein. GPX – mol of GSH consumed/min/mg protein.GST – mol of CDNB-GSH conjugate formed/min/mg protein.GR – mol of NADPH oxidized/min/mg protein.

3.4. Effect of bromadiolone on kidney DNA fragmentation

Figure 3 shows the level of DNA damage in the kidney of control and experimental rats. Rat groups were fed with BDL for 24 hrs (Lane 3), 48 hrs (Lane 4), 72 hrs (Lane 5), 96 hrs (Lane 6), and 120 hrs (Lane 7) shows 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) showed no DNA damages when compared with control rats.

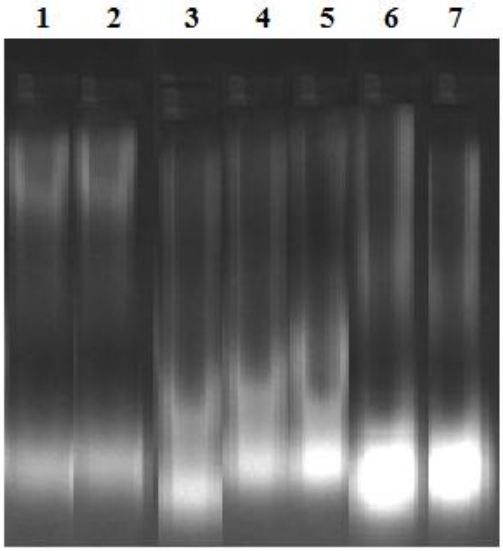

Fig. 4

Fig. 4. Effect of bromadiolone on kidney 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

3.5. Effect of bromadiolone on histopathology

Figure 4 shows a representative photomicrograph of an intact rat kidney from the control group (Fig. 4A). The histoarchitecture pattern of kidney was almost normal in rats fed with BDL 12 hrs (Fig. 4B). The kidney cells exposed to BDL for 24 hrs (Fig. 4C), 48 hrs (Fig. 4D), 72 hrs (Fig. 4E), showed tubular cell necrosis, tubular lumen dilation, dearrangement of basement membrane. Similarly 96 hrs (Fig. 4F) and 120 hrs (Fig. 4G) treated BDL showed intraluminal swelling, loss of proximal tubular cells, and interstitial inflammatory cell infiltration, tubular degeneration, glomerular spaces, vacuolization, medullary congestion, apical blebbing and decreased cellularity of the glomeruli in rats.

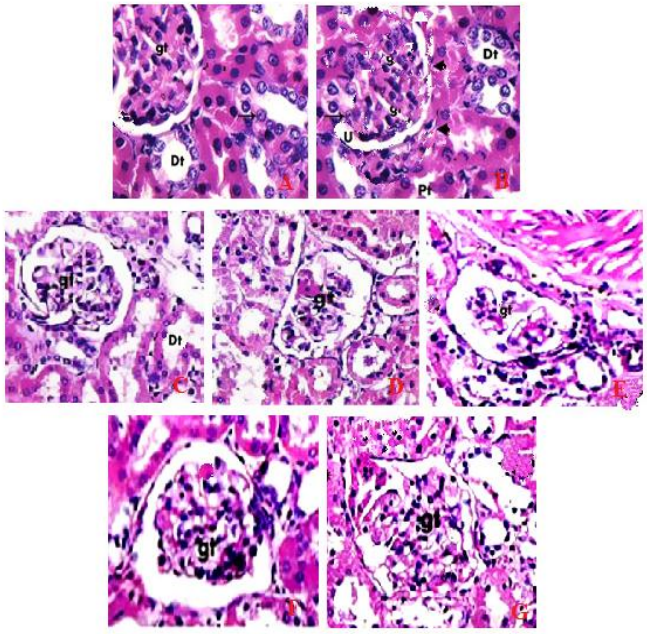

Fig. 5

Fig. 5 Representative photomicrographs from kidney sections of control and experimental rats stained with haematoxylin and eosin. (A) Section of control kidney showing normal arrangement of glomerulus (G) with cytoplasm. (B) Section of kidney fed with BDL for 12 hrs showing the similar histoarchitecture pattern (C) Section of kidney fed with BDL for 24 hrs showing tubular cell necrosis, dearrangement of basement membrane. (D) Section of kidney treated with BDL for 48 hrs showing tubular luman dilation with dearrangement of glomerulus. 72 hrs showing loss of proximal tubular cells (V). Similarly 96 hrs (Fig. 4F) and 120 hrs (Fig. 4G) treated BDL showed intraluminal swelling, and interstitial inflammatory cell infiltration. Magnification ×40.

Discussion:

Chemical control by rodenticides is the most widely used and efficient method of all the available methods for the control of rodent pests both under agricultural and commensal situations [32-33]. A number of highly toxic substances like strychnine, zinc phosphide, barium carbonate, red squill and bromethalin have been commonly used for the control of rodents [34]. The first and second generation anticoagulant rodenticides, the rodent control strategies have undergone a complete change. Nowadays anticoagulant rodenticides *viz.* bromadiolone, brodifacoum and warfarin are becoming more popular for rodent control.

In the present study we demonstrate that aministration of an anticoagulant bromadiolone (0.005%) at various time interval (12 hrs-120 hrs) to male rats *B. bengalensis* (100gm/kg/BW) wheather caused renal toxicity or not. Renal injury confirmd by measuring the serum and urinary levels of urea, uric acid, creatinine, and the creatinine clearance were used as early indicators of renal dysfunction [35]. This is the first report demonstrating on the BDL induced renal toxicity by measuring the urinary

and serum markers in *B. bengalensis*. Our data revealed that the administration of BDL significantly increased urea, uric acid, creatinine, and decresed creatinine clearance in kidney. This may be due to toxic effects of BDL damaged the hypertrophied glomeruli, destructive of its lining epithelia by which increase and decrease the urinary markers. Earlier studies on BDL performed by [11] reported that the administration of BDL damages the kidney of *Mus musculus* at different time intervels. Similary, [36] also reported that Oseltamivir phosphate administration severly damage the kidney tissue in rats. Our study results also revealed the previous report findings that the administration of BDL significantly damge the physiological functions of kidney.

Lipid peroxidation markers such as TBARS, LOOH, CD, and PC content were significantly increased in the BDL intoxicated rat kidney which are in line with the previous report of [37]. The status of lipid peroxidation markers altered certain endogenous radical scavengers is taken as direct evidence for, oxidative stress affecting functional as well as structural integrity of cell and organelles membrane [38-39]. This results suggest that the administration of BDL caused severe damage in the membrane lipids of renal cells due to over production of ROS/RNS led to increase the lipidperoxidative markers. This could be due to BDL induced ROS disturbance to the redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA.

Diminished levels of antioxidants indicate oxidative stress lead to damage of macromolecules in the renal tissue [40]. In the present study, we observed that the administration of BDL decreased non enzymatic antioxidant levels in the kidney of *B.bengalensis*. This finding results were agree with the previous report of [41] who reported that the administration of BDL significantly decreased the non-enzymatic antioxidants in rats. This might be due to the accumulation of ROS induced by BDL can decrease an organism's fitness and cause oxidative damage in the renal tissue through which decreased the non-enzymatic antioxidants.

Enzymatic antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals but enzymatic antioxidant such SOD, CAT, and GPx is playing important role in the protection of body organs from different poisons [36]. In the present study we observed that the aministration BDL treated rat showed significant decreased levels of enzymatic antioxidant in rat kidney. This could be due to toxicity of BDL blocking of vitamin K epoxide, which cannot activate clotting proteins [14] thus lead to increase ROS production in the body which does not converts vitamin K epoxide back to active vitamin K via the enzyme vitamin K epoxide reductase. BDL inhibit this enzyme, resulting in a lack of active vitamin K lead to decreased status of enzymatic antioxidant levels in the kidney.

In the present study we observed that, the BDL intoxicated rats shows severe DNA damage in the renal tissue of *B. bengalensis*. This results showed BDL toxic impact on DNA of renal tissue. This may be due to BDL induced ROS remove one electron and become highly reactive unpaired molecules. This reaction happen through a three-step chain reaction, water is sequentially converted to hydroxyl radical (-OH), hydrogen peroxide (H_2O_2), superoxide radical (O_2-) and ultimately siglet oxygen (O_2). The hydroxyl radical is extremely reactive that immediately removes electrons from any molecule in its path, turning that molecule into a free radical and so propagating a chain reaction. But hydrogen peroxide is actually more damaging to DNA than hydroxyl radical since the lower reactivity of hydrogen peroxide provides travel into the nucleus of the cell, subsequently wreaking have on macromolecules such as DNA. Our report clearly indicat that the BDL damge the renal DNA molecule in *B. bengalensis*.

Histopathological study on rat kidney also support our biochemical and molecular findings that a significant changes occurd in the BDL intoxicated rat when compared with control rats. The histopathology observations of BDL-treated rats showed tubularnecrosis, tubular degeneration, hemorrhage, inflammatory infiltration, swelling of tubules and vacuolization in the renal tissue. This could be due to the accumulation of BDL induced free radicals as a consequence of increased lipid peroxidation and protein carbonyls leads to loss of membrane integrity and other pathological alterations in the kidney tissue.

In conclusion, the present study demonstrated that, BDL has a powerfull rodenticide that control rodent population via producing ROS cytotoxicity in rats. Overall, the findings of the study suggest the potential of BDL in the renal toxicity of *B. bengalensis* by way of affecting normal physiological functions.

Acknowledgements:

The authors acknowledge the Professor and Head, Department of Zoology, Annamalai University, for providing laboratory and chemical facilities to carry out this research works.

References:

1. Elias DJ. Overview of rodent problem in developing countries. FAO, Boletin Fitosanitario 1988; 36, 107–110.
2. Singleton GR, Sudarmaji, Jacob J, Krebs CJ. Integrated management to reduce rodent damage to lowland rice crops in Indonesia. Agriculture, Ecosystem and Environment 2005; 10, 75–82. Timm, R.M., 1986. Construction techniques.
3. Stenseth NC, Herwig L, Skonhoft A, Davis SA, Pech RP, Andreassen HP, Singleton GR, Lima M, Machangu RM, Makundi RH, Zhang Z, Brown PR, Shi D, Wan X. Mice, rats, and people: the bio-economics of agricultural rodent pests. 2003; Front. Ecol. Environ. 1, 367–375.
4. Cao D, Hart K, Pimentel D. Post harvest food losses (vertebrates).In: Pimentel D, editor. Encyclopedia of pest management. 2002; Boca Raton: CRC Press; p. 648-649.
5. Redhead T D, Prevention of plagues of house mice in rural Australia. 1988; In Rodent Pest Mannagemenl (Edited by Prakash I.), .p p. . 191.-205. CRC Press. Boca Raton. FL.
6. Singleton GR, and Petch DA, A review of the biology and management of rodent pests in Southeast, 1994;.
7. Ishizuka M, Tanikawa T, Tanaka KD, Heewon M, Okajima F, Sakamoto KQ, Fujita S, Pesticide resistance in wild mammals e mechanisms of anticoagulant resistance in wild rodents. 2008; J. Toxicol. Sci. 33, 283-291.
8. Pelz HJ, Rost S, Hunerberg M, Fregin A, Heiberg AC, Baert K, MacNicoll AD, Prescott CV, Walker AS, Oldenburg J, Muller CR, The genetic basis of resistance to anticoagulants in rodents. 2005; Genetics 170, 1839-1847.
9. Buckle AP. Rodenticitdal-Their role in rodent pest management in tropical agriculture. In: Singleton, G.R., Hinds, L.A., Leirs, H., Zhang, Z. (Eds.), Ecologically Based Management of Rodent Pests. 1999; Australian Centre for International Agricultural Research, Canberra, Australia.
10. Mulungu LS, Makundi RH, Massawe AW. Characteristics of rodent damage to major crops in Tanzania and crop loss assessment techniques. In: Makundi R.H. (Ed.), Management of Selected Crop Pests in Tanzania. 2006 Tanzania Publishing House Limited, Dar Es Salaam, Tanzania.
11. Revathi K, and Yogananda M. Effect of bromadiolone on haematology, liver and kidney in Mus musculus. 2006; Journal of Environmental Biology January, 27(1) 135-140.
12. Ivan V, Rumen B, Veska Y, Yordan N. Anticoagulant Rodenticide Intoxication in Animals - A Review. 2008; Turk. J. Vet. Anim. Sci.; 32(4): 237-243.
13. Samama MM, Gerotziafas GT, Elalamy I, Horellou MH, Conard J. Biochemistry and clinical pharmacology of new anticoagulant agents. Pathophysiol. 2002; Haemost. Thromb. 32, 218-224.
14. Mount ME, Kim BU, Kass PH. Use of a test for proteins iduced by vitamin K absence or antagonism in diagnosis of anticoagulant poisoning in dogs: cases (1987-1997). 2003; J. Am. Vet. Med. Assoc. 222, 1070-1071.
15. Mundey JS, Thompson LJ. Brodifacoum toxicosis in two neonatal puppies. 2003; Vet. Pathol. 40, 216-219.
16. Radi ZA, Thompson LJ. Renal subcapsular hematoma associated with brodifacoum toxicosis in a dog. 2004; Vet. Hum. Toxicol. 46, 83-84.
17. Binev R, Petkov P, and Rusenov A. Intoxication with anticoagulant rodenticide bromadiolone in a dog - a case report. 2005; *Veterinarski Arhiv 75* (3), 273-282,.
18. Meister RT, Berg GL, Sine C, Miester S, and Poplyk J. eds. Farm Chemical Handbook. 70th ed. Meister Publishing co., Willoughby, OH. 1984;
19. Niehaus WG, and Samuelsson B. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. 1968; Eur. J. Biochem. **6**: 126–130.
20. Jiang ZY, Hunt JY, and Wolff SP Detection of lipid hydroperoxides using the Fox reagent. 1992; Anal. Biochem. 202: 384–389.
21. Levine RL, Garland D, Oliver CN, Amic A, Climent I, Lenz AG, Ahn BW, Shaltiel S, and Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. 1990; Methods Enzymol. 186: 464–478.
22. Moron MS, Deflere JW, and Mannervick B. Levels of glutathione, glutathione reductase and glutathione-*S*-transferase activities in rat hung and liver. 1979; Biochem. Biophys. Acta, 585: 67–78.
23. Ellman GL. Tissue sulphydryl groups. 1959; Arch. Biochem. Biophys. 82: 70–77.
24. Omaye ST, Turbull TD, and Sauberlich HC. 1979; Selected method for the determination of ascorbic acid in animal cells, tissues and fluids. *In* Methods in enzymology. *Edited by* D.B. McCormic and D.L. Wright. Academic Press, New York. pp. 3–11.
25. Desai ID. Vitamin E analysis method for animal tissues. 1984; Methods Enzymol. 105: 138–143.
26. Kakkar P, Das B, and Viswanathan PN. A modified spectroscopic assay of superoxide dismustase. 1984; Indian J. Biochem. Biophys. 21: 130–13
27. Sinha AK. Colorimetric assay of catalase. 1972; Ann. Biochem. 47: 389–394.
28. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DC, and Hoekstra WG Selenium: biochemical roles as a component of glutathione peroxidase. 1973; Science, 179: 588–590.
29. Habig WH, Pabst MJ, and Jakoby WB. Glutathione-*S*-transferase: the first step in mercapturic acid formation. 1974; J. Biol. Chem. 249: 7130–7139.
30. Horn HD, and Burns FH. Assay of glutathione reductase activity. *In* Methods of enzymatic analysis. 1978; *Edited by* H.V. Bergmeyer. Academic Press, New York, pp. 142–146.
31. Hebert MJ, Takano T, Holthofer H, and Brady HR. Sequential morphologic events during apoptosis of human neutrophils. Modulation by lipoxygenase derived eicosanoids. 1996; J. Immunol. 157: 3105–3115.
32. Prakash I, and Mathur RP. Management of Rodent Pests. 1987; 1-133, ICAR Publications
33. Chopra G, Kaur P, and Guraya SS. Predominant rodent species in India. In : R Chand and Co.(ed) Rodents: Ecology, Biology and Control. 1996; 3-19, New Delhi
34. Fishel FM. Pesticide toxicity profile: Miscellaneous rodenticides. http://edis. 2005; ifas. ufl. edu PI-78
35. Thangapandiyan S, and Milton prabu S. Epigallocatechin gallate supplementation protects against renal injury induced by Fluoride intoxication in rats: Role of Nrf2/HO-1 signaling". 2014; Toxicology reports 1 ,12–30.
36. Mohamed HG, Tantawi EL. Histological and histochemical changes of liver, kidney and testis of albino rat after experimental application of oseltamivir phosphate. 2007; (Tamiflu). JASMR, 2(1):35 -43

37. Maddawy El, and Ashmawy El. Hepato-Renal and Hematological Effects of Diclofenac Sodium in Rats. 2013; Global Journal of Pharmacology 7 (2): 123-132.
38. Khan MR and Ahmed D. Protective effects of Digera muricata (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. 2009 Food and Chemical Toxicology, 2(47): 1393-1399.
39. De Leve LD, Wang X, Kuhlenkamp JF, and Kaplowitz N. Toxicity of azathioprine and monocrotaline in murine sinusoidal endothelial cells and hepatocytes: the role of glutathione and relevance to hepatic venoocclusive disease. 1996; Hepatology, 23: 589-599.
40. Nabavi SM, Habtemariam S, Nabavi SF, Sureda A, Daglia M, Moghaddam AH, Amani MA. Protective effect of gallic acid isolatedfrom Peltiphyllum peltatum against sodium fluoride-induced oxida-tive stress in rat's kidney. 2013; Mol. Cell. Biochem. 372 233–239.
41. Kocher DK, and Navjot K. Synergistic effect of bromadiolone and cholecalciferol (vitamin D3) against house rat, *Rattus rattus*. 2013; *International* Journal of Research in BioSciences Vol. 2 Issue 1, pp. (73-82), January.

.

Cite this article as:
N. Sridhar, J. Baskaran, S. Thangapandiyan, S. Dhanasekaran, D. Vasantharaja, S. Mahesh babu. Bromadiolone-Second generation anticoagulant rodenticide induced nephrotoxicity on lesser bandicoot rat *Bandicota bengalensis* (Gray and Hardwicke). Asian Journal of Pharmacology and Toxicology, 03(11), 2015, 13-21.