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RESEARCH ARTICLE

Toxicological Effects of Brodifacoum and Food Energy Inhibitor on Some Physiological Parameters in House Rats (*Rattus rattus*)

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ABSTRACT

This study was conducted to investigate the effects of two different rodenticides, i.e., brodifacoum (BRD) and food energy inhibitor (FEI) on some physiological parameters in house rats (*Rattus rattus*). A total of 42 rats were randomly divided into 3 equal groups. One group served as a control while other two groups were fed BRD and FEI. Results revealed that feed intake and body weights were significantly lower in FEI group as compared with the control group. However, liver, heart and kidney weights were significantly high in BRD group. Mean concentration of glucose and triiodothyronine (T_3) concentrations were significantly low in BRD group, while alanine aminotransferase (ALT), aspartate aminotransferase (AST), thyroxine (T_4), LDH (lactate dehydrogenase) and CK-MB (creatinine-kinase) concentrations were significantly high in the rats of BRD group. It was concluded from this study that BRD has significant toxic effect on physiological biochemistry of house rats as compared to FEI.

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INTRODUCTION

Rodents have long been commensal with humans and they are enormously successful because of their impressive reproductive rates, omnivory and specialized adaptations for gnawing. Annually, they spoil or destroy billions of dollars worth crops, eggs, hatchlings, etc (Mason and Littin, 2003). Rodents are also controlled to prevent damage to buildings and to inhibit the spread of diseases such as salmonellosis, fowl cholera, Weil's disease, bubonic plague and many others (Gage and Kosoy, 2005). Currently, there are two different methods of pest control, chemical and non-chemical methods. Non-chemical methods stress cultural, mechanical and biological controls as well as the strict avoidance or limited use of chemical baits (Ling, 2005). These methods include trapping, shooting and the use of predatory organisms. Despite the availability of alternative methods of pest control, the use of chemicals plays an important role in integrated pest management.

Brodifacoum (3- [3-(4' - bromobiphenyl-4-yl) -1, 2, 3,4- tetrahydro-1-naphthyl] -4- hydroxycoumarin) is an anticoagulant vertebrate pesticide used worldwide to control a variety of rodents that share habit and food sources with human, particularly house rats (Donlan *et al.*, 2003). BRD is a potent, second-generation, anticoagulant rodenticide developed in the mid-1970s and is favored for the control of these pests because of its potency and effectiveness (Eason *et al.*, 2002). Brodifacoum interferes with normal blood clotting by preventing vitamin K recycling which prevents the conversion of inactive precursors into active vitamin K-dependant blood-clotting factors (Littin *et al*., 2000). The normal daily damage to blood vessels is then no longer repaired so that animals die principally from blood loss and its sequelae respiratory or kidney failure (Mason and Littin, 2003). On the other hand, food energy inhibitor (cellulose) based rodenticide, formed of powdered corn cobs, is free of chronic developmental, reproductive and nervous toxicity. Moreover, negligible risk to environment is associated with it. It has been demonstrated that this rodenticide retains water in the gut, resulting in disruption of water transport through the gut wall, in turn interferes with normal digestion of the rodent (Mason and Littin, 2003).

To the best of our knowledge, the comparative study of these rodenticides on serum biochemistry is very sparse. Therefore, this study was planned to investigate the effect of these rodenticides on some biochemical parameters of house rats (*Rattus rattus*).

MATERIALS AND METHODS

The present study was conducted on 42 house rats (*Rattus rattus*) in District Faisalabad, Pakistan. To capture rats, 18 villages were randomly selected under the defined area. Indoor population of these rats was assessed by active signs of damage to the materials, railway tracks, existence of burrows and rodent droppings scattered on the site. When the required numbers of rats were captured, they were shifted to Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. These were weighed and randomly assigned to different metallic cages. Total of 42 rats were divided into 3 equal groups having 7 male and 7 female rats in each group.

One group was treated with cellulose (FEI), second group was fed BRD and third group was kept as control. Cellulose was fed in the form of pellets according to the body weight (an average of 20 g of pellets). BRD was fed in the form of blocks containing 0.05% active ingredient. Whereas, the control rats were given the measured normal feed. The experiment was lasted for 14 days. Feed intake and body weight of individual rats was measured on daily basis. At the end of experiment, rats were decapitated and blood was collected without anticoagulant and subjected to extraction serum and stored at -20°C till analysis. Body organs including heart, liver, kidney, ovary, uterus and testes were removed and weighed.

For the quantitative measurement of glucose, commercially available kit (Bioray®; CAT # 1426-6) was used. Commercial kits were also used for the quantitative determination of AST & ALT (Randox® CAT # AS147 & AL146), CK-MB (Spinreact®; CAT # 41254) and LDH (Spinreact®; CAT # 41220). Similarly, triiodothyronine (T_3) and thyroxine (T_4) concentrations were determined by commercially available kits (Randox®; CAT # BC-1005 and BC-1007, respectively).

The data obtained was subjected to statistical analysis as described by Steel *et al*. (1997). In case of significant difference between different groups Duncan Multiple Range Test was applied (Duncan, 1955).

RESULTS

Feed consumption of male and female rates was significantly ($P \leq 0.05$) low in FEI group whereas body weight was significantly reduced in FEI treatment as compared to the control (Table 1). However, liver, heart and kidneys weights in both the sexes were significantly ($P \leq 0.05$) higher in BRD groups as compared to control and FEI groups. Mean testes, uterus and ovary weight did not differ between the treatments (Table 1).

When gender based interaction of various treatments analyzed, it was observed that glucose concentration was significantly low in FEI group. Mean concentrations of ALT, AST, T_4, LDH and CK-MB were significantly higher ($P \leq 0.05$) in BRD treated male and female rats whereas T3 concentration was significantly low ($P \leq 0.05$) in BRD group (Table 2).

DISCUSSION

Toxicological studies of BRD and FEI on serum biochemistry is extremely sparse. This research work was conducted to compare the effect of BRD and FEI on feed intake, body weight, organs weight and serum biochemistry of house rats. We found that feed consumption was lower in FEI treated group as compared to control. This may be due to the interference of FEI with the digestive system by blocking the intestines and cecum (Mason and Littin, 2003). In this experiment it was observed that body weight of FEI fed group was significantly lower compared to control group. The lower body weight could be attributed to low feed intake of FEI group which is ascribed to the mechanism of action of this compound through blocking the digestive tract. On the other hand, BRD depressed the rodents for several days and then caused severe blood flow through mouth in the latter stages (Littin *et al*., 2000) due to which they may not eat properly and resulted in less feed intake. BRD inhibits the generation of the active form of vitamin K via the inhibition of vitamin K reductase. When vitamin K cannot be regenerated, clotting factors are not activated which ultimalty results in the death of the rodents (Burkhart, 2000). Therefore, we can conclude the body weight of the rats was proportion to the feed intake of their respective groups.

Table 1: Feed intake, body weight and organ weight in *Rattus rattus* fed food energy inhibitor (FEI) and brodifacoum (BRD)

Parameters (g)	Control	FEI	BRD
Male			
Feed consumption/day	15.56±1.55^{a}	10.44±0.89^{b}	12.11±0.45^{ab}
Body weight	115.56±3.45^{a}	80.43±2.33^{c}	89.67±3.21^{b}
Liver	3.7±0.02^{b}	3.8±0.04^{b}	4.1±0.02^{a}
Heart	0.38±0.001^{c}	0.50±0.001^{b}	0.56±0.002^{a}
Kidneys	0.60±0.001^{c}	0.9±0.002^{b}	1.2±0.001^{a}
Testes	1.89±0.03	2.23±0.02	1.98±0.01
Female			
Feed consumption/day	13.56±1.33^{a}	9.81±0.78^{b}	11.99±0.66^{ab}
Body weight	113±10.73^{a}	62.05±5.85^{c}	72.89±9.19^{b}
Liver	2.1±0.01^{c}	3.6±0.01^{b}	4.00±0.01^{a}
Heart	0.27±0.001^{c}	0.41±0.001^{b}	0.54±0.002^{a}
Kidneys	0.45±0.001^{c}	0.91±0.002^{a}	1.34±0.002^{a}
Uterus	0.05±0.001	0.04±0.001	0.05±0.001
Ovary	0.02±0.002	0.03±0.002	0.04±0.001

Values (Mean±SE) with different superscripts in a row differ significantly ($P<0.05$).

In this study, we also observed that weight of liver, kidney and heart were significantly increased in BRD group. Eason *et al.* (2002) found that in male rats fed 0.25 mg/kg/day sodium monofluroacetate, a second generation rodenticide like brodifacoum, the heart, liver and kidney weight were significantly increased in Sprague-Dawley rats. These results are quite consistent to our findings; therefore, we can speculate that the increased weight of these vital organs could be due to inflammation and accumulation of toxic substances in these organs which are the major sites of toxicity (Eason *et al*., 2002).

The studies of biochemical parameters have significant value in toxicological evaluations as alterations appear quite before the clinical symptoms produced by the toxicants (Rao, 2006). In this study, we found that concentration of glucose markedly decreased in FEI group. The simple explanation to this decrease could be that rats consumed less feed, therefore, the levels of glucose in serum of both groups decreased. In this study,

Table 2: Various biochemical parameters in rats treated with food energy inhibitor and brodifacoum

Parameters	Control		Food energy inhibitor		Brodifacoum	
	Male	Female	Male	Female	Male	Female
Glucose (mg/dL)	155.33±2.12a	147.54±1.23a	68.76±1.45c	65.44±1.56c	120±3.22b	117.32±1.32b
ALT (U/L)	11.23±0.99c	12.76±0.98c	32.44±1.22b	33.22±1.45b	46.78±2.11a	45.331.55a
AST (U/L)	20.78±1.11a	19.22±1.34c	22.44±2.01ab	21.32±1.90b	26.56±1.11a	29.88±1.98a
T_3 (ng/mL)	5.55±0.57a	4.99±0.33a	5.11±0.76a	5.15±0.98a	3.56±0.66b	3.78±0.24b
T_4 (µg/mL)	4.98±0.77b	4.78±0.11b	5.89±0.45b	5.73±0.34b	7.61±0.98a	7.59±0.66a
LDH (U/L)	20.22±1.11b	22.34±1.99b	25.54±0.99b	23.98±0.78b	45.78±1.23a	47.98±0.99a
CK-MB (mg/dL)	138.45±2.34c	143.22±2.13c	245.33±4.54b	260.56±3.22b	578.13±2.33a	567.12±4.23a

Values (Mean±SE) with different letters in a row differ significantly (P<0.05).

concentration of ALT and AST increased significantly in BRD group. The increased level of ALT is more sensitive marker to measure hepatotoxcity (Rao, 2006). We can conclude that liver damage liberated liver enzymes into the blood and resulted in elevated level of ALT and AST.

LDH along with AST and ALT are mainly used to the evaluation of hepatic toxicity of liver (Rao, 2006). According to Dere *et al.* (2007), when the membrane of hepatocyte is damaged, the contents inside the cells leak out into the blood including LDH. In this study, the level of LDH was significantly high in BRD group, indicating liver damage of the rats.

Normal T_3 and T_4 level is necessary for normal growth, development and maintenance of body functions. Brown (2003) reported that countless number of chemicals affect production, transport and metabolism of thyroid hormones. Lacasana *et al*. (2010) reported that environmental poisons may interfere with thyroid gland functions through different mechanisms of actions, e.g., at the receptor level, in binding to transport proteins, in cellular uptake mechanisms or modifying the mechanisms of thyroid hormones. In our study, T_3 concentration was significantly low while T_4 was significantly high in BRD group. Similar to our findings, Zaidi *et al*. (2000) reported that as a result of chemical insult, the level of T_3 is suppressed while T_4 level is slightly elevated. Tseng and Chen (1992) concluded that drugs intoxication directly affects thyroid activity reducing T_3 activity or convert T_3 into T_4 and increased its level.

In the present study, CK-MB was significantly high in BRD fed group. CK-MB is a dimeric enzyme which catalyzes the transfer of high-energy phosphate between ADP and creatine (Mitani *et al*., 2000). This enzyme has been reported to be elevated in myocellular necrosis, severe ischemia, myocardial infarction and causes increased mortality (Ricciardi *et al*., 2001; Landesberg *et al*., 2003). In out study, the elevated level of CK-MB might be due to the intoxication of BRD in heart tissues.

We can conclude that BRD and FEI caused significant alterations in serum physiology of house rats, however, the effects were more pronounced in BRD group compared to FEI.

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