RESEARCH ARTICLE

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Corresponding Author
Kanan Saxena
Parasitology and Toxicology Research Laboratory, Post Graduate Department of Zoology, Govt. Meera Girls College, Udaipur - 313001 (Rajasthan), India.
Email:kanansaxena@yahoo.com

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Biochemical response of Indian Desert Gerbils to Difethialone Anticoagulant, Under Laboratory Conditions.

Kanan Saxena
Parasitology and Toxicology Research Laboratory, Post Graduate Department of Zoology, Govt. Meera Girls College, Udaipur - 313001 (Rajasthan), India.

ABSTRACT
Difethialone, a second generation anticoagulant shows a very good efficacy against Indian Desert Gerbil, *Meriones hurrianae*, as seen in the preliminary toxicity tests performed. Biochemical studies in laboratory reveal that the administration of Difethialone (LD_{50}) has significant toxic effects on the hepatic tissues of desert gerbils. Liver is the chief organ which metabolizes the rodenticide. Hence, different liver function tests were performed on liver residues at different intervals of days to analyse Difethialone toxicity. Also, the associated symptoms of poisoning were observed. A quick change in liver parameters with a very small dose resulting in higher mortality of gerbils was seen. Therefore, Difethialone emerges as a promising and comparatively cheaper substitute in rodent pest control.

Key words: Biochemical, Difethialone, Liver function tests, *Meriones hurrianae*, Toxicity.

INTRODUCTION

The range of currently available acute rodenticides is quite narrow due to their high toxicity. New generation rodenticides are mostly the anticoagulant rodenticides. Anticoagulants are widely used and are highly effective in controlling nuisance rodent populations. They are commonly available as cereal-based baits to make them palatable to targeted species. After their uptake the animal dies because of haemorrhage into body cavities which is not supposed to be painful and simulates natural death. Also, these anticoagulant rodenticides are a common cause of poisoning in both domestic and wild animals, either by direct ingestion of the bait or relay toxicosis due to the consumption of poisoned rodents[1]. The repeated use of first generation rodenticides has resulted in resistance to these substances in rats and mice, thus leading to the development of second-generation rodenticides: bromadiolone, brodifacoum and Difethialone [2, 3]. The onset of toxic effects of second generation anticoagulants is slow, hence the baits can be prepared in very low concentrations of the toxicant [4]. These second-generation rodenticides have a prolonged efficacy because of their strong binding to target enzymes that are highly lipophilic proteins [5]. These toxins are rapidly absorbed from the gastrointestinal tract following oral exposure.

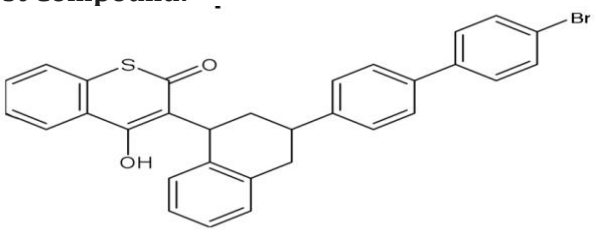
Anticoagulant rodenticides exert their effect by inducing a vitamin K-dependent coagulopathy leading to uncontrolled haemorrhage and death.

The objective of this study is to measure the efficacy of Difethialone in rat liver following the LD_{50} dose. The tendency for anticoagulants to persist in mammalian liver is influenced by the magnitude of the dose ingested and the relative affinity of the compound for receptors in the liver, which determine the hepatic elimination half-life and the proportion of the dose retained[5]. Accordingly, liver tissue is the focus for most investigations of anticoagulant persistence.

MATERIAL AND METHODS

The Indian desert gerbil, *Meriones hurrianae* was used as the test species. Adult gerbils of both the sexes were housed in polypropylene cages in a controlled temperature environment (18°C ± 2°C). Gerbils were acclimatised for at least 10 days prior to starting the trial, and throughout the trial had free access to water and palatable diet (Rat feed: Hindustan Lever Ltd., New Delhi, India).

Test Compound:



Difethialone

Chemical structure of Difethialone

The acute median lethal dose LD_{50} (0.28 mg/kg body weight) of Difethialone anticoagulant was administered orally to the animals with the aid of a stomach gavage needle with a ball tip no. 16 according to the body weight of the animals. Later the animals were provided with the normal diet. All the gerbils were keenly watched every day for poisoning symptoms.

Various parameters for liver tissue biochemistry were estimated in the gerbils, before and after the administration of the poison dose. The animals were autopsied at set time intervals of 2, 4, 6 and 8 days after the administration of poison dose and examined for the changes in the liver tissue. These changes were compared with their respective vehicle controls. The systronic photoelectric colorimeter 101 was used for biochemical analysis.

Quantitative estimation of Glycogen in liver tissue was done by Rex Montgomery method[6]

Liver cholesterol was quantitatively estimated by Zlatkis et. al. method [7] .

Protein assay in the liver of gerbils was carried out by Lowry et. al. method [8].

Statistical Analysis:

The data are expressed as means ± SE. The significance of the differences in mean values among control and the treated groups was evaluated using Student's t-test.

RESULTS AND DISCUSSION

High toxicity of Difethialone was observed with the onset of poisoning symptoms within 2-3 days. Loss of appetite followed by uneasiness and pulmonary distress was a prominent feature in all the treated gerbils. Traces of blood were observed in the ears, nose and conjunctiva of the eye in some animals. The liver turned pale progressively and numerous yellowish white blisters appeared on its surface. Subcutaneous haemorrhage was noted.

The study of biochemical parameters is significant in toxicological evaluations as alterations appear quite before the clinical symptoms produced by the toxicants [3]. Tissue parameters viz. Liver Glycogen, Liver Cholesterol and Liver Protein were studied for toxic effects of Difethiaone. Non significant ($P > 0.05$) difference was observed between the normal control and vehicle control groups of gerbils. A decline in liver glycogen, cholesterol and protein was observed at 2nd, 4th, 6th and 8th day of autopsy interval after Difethialone poisoning as shown in Table 1, 2 and 3.

The main site of action and accumulation of anticoagulant rodenticides is the liver, thus making it a diagnostically useful post-mortem tissue [9]. Glycogen is formed in the liver and muscles, and anticoagulants cause severe disfunctioning in carbohydrate metabolism as the hepatic cells undergo intoxication stress. When blood sugar tends to fall, glycogen is converted into glucose and mobilised in the blood stream resulting in hyperglycemia. This indicates glycogenolysis in hepatic parenchymal cells. Decreased glycogen levels may be due to reduced glycogenesis. Possibly the glycogen is utilized more following the drug injury.

Liver plays a key role in cholesterol metabolism as cholesterol is synthesised in the liver[10]. The damage caused to the liver by anticoagulant poisoning disturbs the cholesterol levels as the feedback and caloric mechanisms that govern the cholesterol synthesis in the liver are lost when degenerative changes occur due to acute toxicity.

Statistically significant decline in liver protein level was observed on all autopsy days. After Difethialone intoxication, reduced food intake was noticed in the treated gerbils. This altered nutritional status of the animals seems to be responsible for depleted protein levels. The damage caused by the anticoagulant to the parenchymal cells of the liver results in the decreased protein content.

CONCLUSIONS

The biochemical parameters are the main bioindicators exhibiting the toxic effects of Difethialone. The study suggests that Difethialone appears to be a potent rodenticide affecting liver of gerbils.

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Autopsy days	Liver Glycogen (mg/gm)		
	NC	VC	T
2	18.98 ± 1.02[d]	19.50 ± 0.76	14.66 ± 0.44[b]
4	20.0 ± 0.28[d]	22.33 ± 0.88	12.50 ± 0.57[c]
6	22.16 ± 0.72[d]	23.50 ± 0.57	4.00 ± 0.28[c]
8	19.33 ± 0.60[d]	21.66 ± 0.92	1.33 ± 0.16[c]

Table 1: Toxicity of Difethialone on Liver Glycogen in *Meriones hurrianae*

Experimental groups:
NC – Normal control group; VC – Vehicle control group; T – LD_{50} treated group
Values are expressed as mean ± S.E.
a: P < 0.05; b: P < 0.01; c: P < 0.001; d: P > 0.05

Autopsy days	Liver Cholesterol (mg/gm)		
	NC	VC	T
2	10.66 ± 0.66[d]	11.33 ± 0.66	9.33 ± 1.33[d]
4	11.33 ± 1.33[d]	12.0 ± 1.15	7.33 ± 0.66[a]
6	10.0 ± 1.15[d]	11.33 ± 0.66	4.66 ± 0.66[b]
8	11.33 ± 0.66[d]	10.66 ± 01.33	2.66 ± 0.66[b]

Table 2: Toxicity of Difethialone on Liver Cholesterol in *Meriones hurrianae*

Experimental groups:
NC – Normal control group; VC – Vehicle control group; T – LD_{50} treated group
Values are expressed as mean ± S.E.
a: P < 0.05; b: P < 0.01; c: P < 0.001; d: P > 0.05

Autopsy days	Liver Cholesterol (mg/gm)		
	NC	VC	T
2	82.954 ± 3.91[d]	84.436± 2.56	74.066 ± 1.95[a]
4	91.842 ± 1.48[d]	87.398 ± 2.96	60.734 ± 0.74[c]
6	88.88 ± 2.56[d]	89.620 ± 1.95	47.402 ± 1.48[c]
8	85.917 ± 2.96[a]	95.546 ± 1.28	28.886 ± 1.28[c]

Table 3: Toxicity of Difethialone on Liver Protein in *Meriones hurrianae*

Experimental groups:
NC – Normal control group; VC – Vehicle control group; T – LD_{50} treated group
Values are expressed as mean ± S.E.
a: P < 0.05; b: P < 0.01; c: P < 0.001; d: P > 0.05

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