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Toxicity Study of Various Leaf Extracts of *Dregea volubilis* [Benth] (DV) and *Leptadenia reticulata* [W&A] (LR)

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Abstract: It is presumed that ayurvedic drugs have lesser side effects as compared to allopathic drugs. For the safety to use these plants and preparations (gel and powder forms), the medicinal plants need to be evaluated for their toxicity. The aim of this study was to test the acute toxicity of two medicinal plants, *Dregea volubilis* and *Leptadenia reticulata* leaves. The acute toxicity study was carried out on Swiss mice with a dose of 2000 mg/kg body weight orally. The single administration of the various extracts of *Dregea volubilis* and *Leptadenia reticulata* on Swiss mice was carried out orally. The observations of changes in body weight, food and water intake as well as cage side observations were noted. The plants were found to be non-toxic.

Key words: *Dregea volubilis* · *Leptadenia reticulata* · Acute toxicity

INTRODUCTION

The use of herbal drugs for the management of certain ailments is unabated in most developing communities due to easy access and for economic reasons. Plants, therefore, remain the main source of the active drugs from a natural source and are still indispensable in the traditional medicine for treating a number of diseases [1]. The traditional indigenous systems of medicine contain active organic compounds and are employed in the treatment of diseases of diverse origins. Traditional medicines are used by about 60% of the world population both in the developing and developed countries where modern medicines are predominantly used [2]. The World Health Organisation (WHO) survey indicates that about 70 – 80 % of the world's population relies on non-conventional medicine, mainly of herbal source in the primary health care [3]. Experimental screening method is, therefore, important to ascertain the safety and efficacy of herbal products as well as to establish the active components of these herbal remedies [4]. To determine the safety of the plant products for human use, toxicological evaluation such as hepatotoxicity, CNS toxicity and renal toxicity should be carried out in various experimental animals to predict the toxicity and to provide guidelines for selecting a 'safe' dose in humans [5]. But it is quite difficult to ascertain certain adverse effects in animals such as headache, abdominal pain, dizziness and visual disturbances. In addition, interspecies differences in the pharmacokinetic parameters make it difficult to translate some adverse effects from animals and human beings. Neverthless, the evaluation of adverse effects of sub-chronic and chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the plant preparation. Acute studies with a range of doses have to be conducted first to select proper dose(s) for chronic and sub–chronic studies; the doses selected for chronic and sub-chronic toxicity studies should be at and above the suggested human dose [6]. *Dregea volubilis* (DV) and *Leptadenia reticulata* (LR) belong to family Asclepiadaceae. DV is widely used in Indian traditional medicines and the leaf paste is used to treat rheumatic pain, cough, fever and severe cold. Leaf paste is taken along with pepper to treat dyspepsia, bark paste mixed with hot milk is used internally for treating urinary infections and leaves are showed hypoglycaemic and hypolipidaemic activity [7]. LR commonly known as Jiwanti, Jiwanti has been claimed to be useful as galactagogue, antibacterial, lactogenic, antifungal, hypotensive, restorative, tonic and leaves are showed hypoglycaemic and hypolipidaemic activity [8]. The present study was carried out to assess the toxicity of various extracts of leaves of DV and LR such as Petroleum

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ether extracts (PEDV, PELR), Ethyl acetate extracts (EADV, EALR) and Ethanolic extracts (ETDV, ETLR). Hence, all these extracts were subjected to acute and sub–chronic toxicity studies to further confirm these activities using animals.

MATERIALS AND METHODS

Preparation of Different Plant Extracts: DV and LR leaves were collected from the forest of Kalakatu, Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India, Palayamkottai. Fresh plant leaves were shade dried at room temperature, ground into fine powder and then extracted (amount 500 g) with solvents such as petroleum ether, ethyl acetate and ethanol by increasing polarity of each solvent at a particular time interval for 24 hours by continuous hot extraction using the soxhlet apparatus at a temperature of 60°C [9]. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

Acute Toxicity Study with Petroleum Ether, Ethyl Acetate and Ethanolic Extracts of DV and LR: Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute and chronic toxicity assays. Acute toxicity assay involved the estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals) [10]. Acute oral toxicity of petroleum ether, ethyl acetate and ethanolic extract of DV and LR was carried out as per the guidelines Organization of Economic Co-operation and Development (OECD – 423) guidelines [7]. The albino mice of 20-25 g were fasted over night and provided only water, after which the extracts were administered by gastric intubations to relevant group of animals orally at the dose of 5 mg/kg body weight in Tween-80. The animals were observed for 14 days and maintained with normal food. Mortality rate of 2 or 3 animals in 14 days were recorded and the dose is said to be toxic. But when mortality of one animal was observed, then the same dose is repeated again for confirmation. However, when mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/kg body weight. Toxic symptoms were observed for 72 hours including behavioural changes, locomotion, convulsions and mortality [11, 12]. The same method was followed for the acute toxicity study of other extracts.

Cage Side Observations: Observations include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behaviour pattern, special attention is directed for the observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

Body Weight, Food and Water Intake: Body weight, food and water intake were recorded at two-day intervals.

Pathology: Surviving animals were fasted overnight, weighed and humanely killed on the 15^{th} day using anesthetic ether. All test animals were subjected to gross necroscopy.

Sub-Chronic Test for Ethanolic Extracts of DV and LR: This experiment evaluated the toxicity potential of DV and LR i.e. this study evaluated the protection of the extracts of DV and LR. Wistar rats (160 ± 10 g) were used for the present study. The animals are divided into seven groups of six animals in each group. The dose of the extract was calculated based on the body weight of the animal. The animals in group I were administered with a single daily dose of 0.5 ml of Tween 80 orally for 20 days. The animals in Group II and III were administered with 100 mg/kg body weight of ethanolic extracts of DV and LR. The animals in Group IV and V were administered with 250 mg/kg body weight of ethanolic extracts of DV and LR. The animals in Group VI and VII were administered with 500 mg/kg body weight of ethanolic extracts of DV and LR respectively for 20 days orally [13]. The animals were then weighed every five days, from the start of the treatment, to record the weight variation. At the end of the treatment, blood samples were collected by puncturing retro orbital plexus after mild anaesthesia for biochemical analysis and the liver tissues were collected for histopathological studies [14]. The collected blood sample was centrifuged within 5 minute of collection at 4000 mg for 10 minute to obtain plasma, which was analysed for total cholesterol, total glyceride, HDL, LDL, plasma glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Statistical Analysis: Data were expressed as mean±SEM. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison tests using Instat-3 software package (Graph pad), Prism Ltd, USA.

RESULTS

Acute Toxicity Study with PEDV, EADV and ETDV: The acute toxicity of petroleum ether (PEDV), ethyl acetate (EADV) and ethanol (ETDV) extract of DV was evaluated using OECD – 423 guidelines. There was no mortality or morbidity observed in animals throughout the 14-day period following single oral administration at all selected dose levels of the PEDV, EADV and ETDV (Table 1). The animals did not show any changes in the general appearance during the observation period. Morphological characteristics such as fur, skin, eyes and nose appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviours such as self mutilation, walking backward and so forth were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was also normal.

Effect of ETDV and ETLR on Body Weight Changes in Rats: A significant change ($P< 0.05$) was observed in the body weight of all the test animals when compared to control. The results are shown in Figure 1 and Table 2, where group I animals were treated with Tween 80 (5 ml/kg), group II and III animals with 100 mg/kg of ETDV and ETLR, group IV and V animals with 250 mg/kg of ETDV and ETLR, group VI and VII animals with 500 mg/kg of ETDV and ETLR.

Effect of ETDV and ETLR on Kidney, Heart, Liver and Brain in Rats: From the study it was clear that significant ($P< 0.05$) changes in the weights of various organs of the animals occurred with higher doses of the extract (500 mg/kg body weight), but macroscopic examinations did not show any changes in colour of the organs of the treated animals compared with the control. The results are shown in Figure 2 and Table 3. Group I animals were treated with Tween 80 (5 ml/kg), group II and III animals with 100 mg/kg of ETDV and ETLR, group IV and V animals with 250 mg/kg of ETDV and ETLR, group VI and VII animals with 500 mg/kg of ETDV and ETLR.

Effect of ETDV and ETLR on Biochemical Profiles of Rats: From the study it was evident that there was significant decrease ($P< 0.05$) in the plasma glucose level in treated rats with a dose of 250 mg/kg and 500 mg/kg when compared with the control rats. Significant decrease ($P< 0.05$) in the plasma total cholesterol (TC), triglyceride (TG) and LDL – cholesterol levels were also observed. But a significant increase ($P< 0.05$) in HDL – cholesterol levels were observed in 250 mg/kg and 500 mg/kg dose treated animals when compared with the control animals. The results are shown in Figure 3 and Table 4.

Table 1: Acute Toxicity Study of PEDV, EADV, ETDV, PELR, EALR and ETLR on Experimental Mice

	Dose (mg/kg)	Sign of Toxicity (ST/NB)	Mortality ($D.S^{-1}$)
Group I	0	0/3	0/3
Group II	300	0/3	0/3
Group III	2000	0/3	0/3

ST – sign of toxicity; NB – normal behaviour; D – died; S – survive. Values are expressed as number of animals (n = 6).

Table 2: Effect of ETDV and ETLR on Body Weight Changes in Rats

Treatment	Day 1	Day 5	Day 10	Day 20
Control	172.01 ± 3.1	173.4 ± 1.17	177.01 ± 1.1	179.3± 2.5
ETDV 100 mg/kg	174.2 ± 0.3	177.3 ± 1.3	181.02 ± 1.7	183.2 ± 0.5
ETLR 100 mg/kg	174.4 ± 0.2	177.4 ± 1.4	182.5 ± 1.3	183.2 ± 0.2
ETDV 250 mg/kg	176.3 ±0.3	180.04 ± 1.5	183.2 ± 1.01	185.2 ± 0.2
ETLR 250 mg/kg	176.1 ± 0.2	181.4 ± 1.1	184.4 ± 1.2	186.6 ± 0.3
ETDV 500 mg/kg	177.2 ± 1.3	182.5 ± 1.8	185.02 ± 0.3*	189.3 ± 1.2*
ETLR 500 mg/kg	177.8 ± 1.4	183.6 ± 1.5	186 ± 0.1*	189.6 ± 1.5*

n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison test. *$P < 0.05$, compared to normal control group.

Table 3: Effect of ETDV and ETLR on kidney, heart, liver and brain of the rats

Treatment	Heart (g)	Kidney (g)	Liver (g)	Brain (g)
Control	0.31 ± 0.02	0.63 ± 0.04	3.23 ± 0.03	0.62± 0.4
ETDV 100 mg/kg	0.32 ± 0.01	0.78 ± 0.03	3.38 ± 0.02	0.62 ± 0.3
ETLR 100 mg/kg	0.32 ± 0.02	0.78 ± 0.02	3.39 ± 0.03	0.63 ± 0.2
ETDV 250 mg/kg	0.33 ± 0.01	0.79 ± 0.03	3.41 ± 0.02	0.71 ± 0.03
ETLR 250 mg/kg	0.32 ± 0.02	0.78 ± 0.04	3.43 ± 0.01	0.71 ± 0.05
ETDV 500 mg/kg	0.34 ± 0.02*	0.82 ± 0.02*	3.75 ± 0.03*	0.73 ± 0.05*
ETLR 500 mg/kg	0.33 ± 0.03*	0.84 ± 0.02*	3.82 ± 0.04*	0.75 ± 0.03*

n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison test. *$P < 0.05$, compared to normal control group.

Table 4: Effects of ETDV and ETLR on Glucose, Cholesterol, Triglyceride, HDL and LDL

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	91.45 ± 0.14	46.62 ± 0.40	34.20 ± 0.15	48.21±0.3	90.02 ± 1.70
ETDV 100 mg/kg	87.45 ± 0.65	32.82 ± 0.04	31.79 ± 0.40	76.3 ± 0.70	87.60 ± 0.10
ETLR 100 mg/kg	87.86 ± 0.52	33.74 ± 0.06	32.33 ± 0.77	74.2 ± 0.77	75.67± 0.22
ETDV 250 mg/kg	83.22 ± 0.54*	29.19 ± 0.05*	27.20 ± 0.56*	84.2 ± 0.88*	54.60 ± 0.50*
ETLR 250 mg/kg	82.69 ± 0.63*	28.18 ± 0.43*	26.33 ± 0.10*	81.4 ± 0.97*	52.50 ± 0.53*
ETDV 500 mg/kg	79.38 ± 0.44*	26. 20 ± 0.90*	23.30 ± 0.01*	86.5 ± 1.20*	39.75 ± 0.43*
ETLR 500 mg/kg	78.60 ± 0.23*	25.43 ± 1.20*	22.43 ± 0.08*	84.6 ± 0.55*	38.55 ± 0.56*

n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison test. *$P < 0.05$, compared to normal control group.

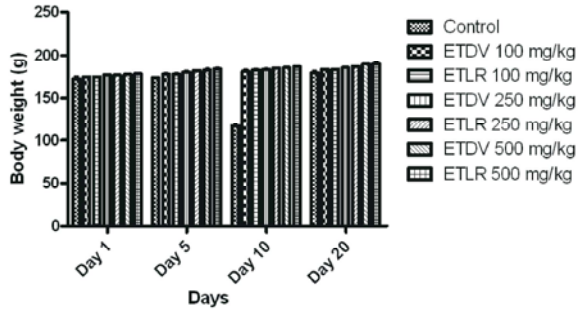

Fig. 1: Effect of ETDV and ETLR on Body Weight Changes in Rats

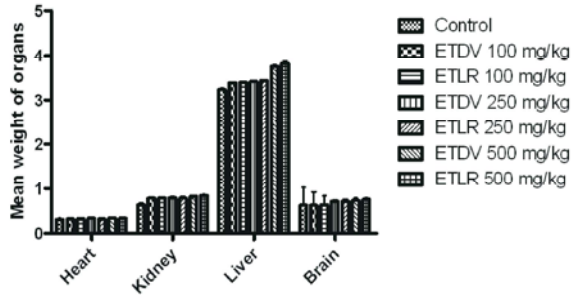

Fig. 2: Effect of ETDV and ETLR on Kidney, Heart, Liver and Brain of the Rats

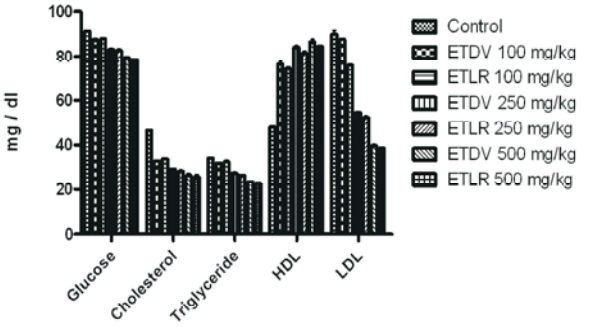

Fig. 3: Effects of ETDV and ETLR on Glucose, Cholesterol, Triglyceride, HDL and LDL

Effect of ETDV and ETLR on Biochemical Parameters Such as AST, ALT and ALP: Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) levels were also normal in the ETDV and ETLR treated animals. From the results of biochemical studies there were no evidence of severe toxicity associated with the administration of higher concentration of ETDV and ETLR. The results are shown in Figure 4 and Table 5, where group I animals were treated with Tween 80 (5 ml/kg), group II and III animals treated with 100 mg/kg of ETDV and ETLR, group IV and V animals

Table 5: Effects of ETDV and ETLR on AST, ALT and ALP

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	334.4 ± 10.47	78.4 ± 7.78	265.60 ± 8.89
ETDV 100 mg/kg	325.4 ± 9.81	76.6 ± 2.16	273.01 ± 2.74
ETLR 100 mg/kg	328.2 ± 8.47	74.4 ± 3.02	267.60 ± 2.80
ETDV 250 mg/kg	319.7 ± 7.70	69.5 ± 2.76	259.76 ± 1.98
ETLR 250 mg/kg	321.2 ± 7.12	67.9 ± 3.54	257.91 ± 2.67
ETDV 500 mg/kg	315.4 ± 7.12*	60.6 ± 2.80*	247.43 ± 1.78*
ETLR 500 mg/kg	309.6 ± 6.06*	59.8 ± 2.97*	245.61 ± 2.33*

n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison test. *$P < 0.05$, compared to normal control group.

Table 6: Effects of ETDV and ETLR on Haematological Parameters (Haemoglobin, RBC and WBC)

Treatment	Haemoglobin (g/dl)	RBC (10^6 / mm^3)	WBC ($5x10^3$–10^4/ mm^3)
Control	12.2 ± 0.30	10.6 ± 0.03	12.3 ± 0.04
ETDV 100 mg/kg	13.6 ± 0.20	10.57 ± 0.05	9.4 ± 0.03
ETLR 100 mg/kg	13.4 ± 0.12	10.32 ± 0.04	8.2 ± 0.05
ETDV 250 mg/kg	12.8 ± 0.20	9.16 ± 0.13	11.3 ± 0.04
ETLR 250 mg/kg	13.6 ± 0.30	9.55 ± 0.45	10.6 ± 0.02
ETDV 500 mg/kg	11.1 ± 0.40*	8.94 ± 0.06*	11.8 ± 0.12*
ETLR 500 mg/kg	11.3 ± 0.25*	9.10 ± 0.33*	11.9 ± 0.09*

n=6. The statistical analysis was carried out using one way ANOVA followed by Dunnett's multiple comparison test. *$P < 0.05$, compared to normal control group

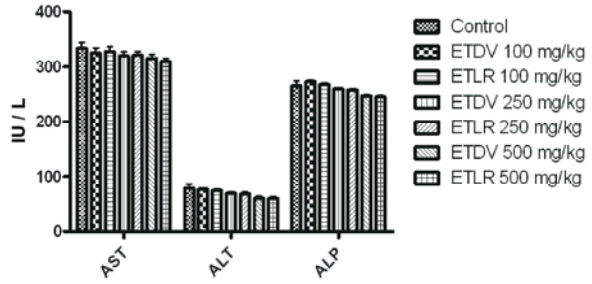

Fig. 4: Effects of ETDV and ETLR on AST, ALT and ALP

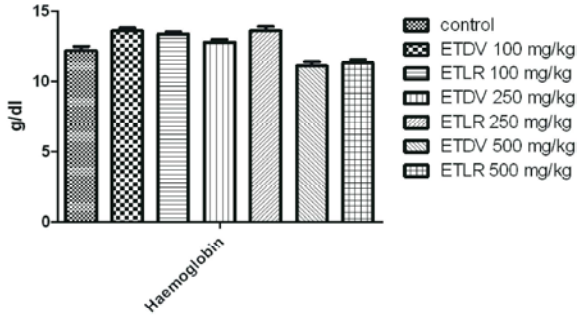

Fig. 5: Effect of ETDV and ETLR on Haematological (Haemoglobin)

treated with 250 mg/kg of ETDV and ETLR, group VI and VII animals treated with 500 mg/kg of ETDV and ETLR. From the study it was evident that, there was a significant decrease ($P < 0.05$) in AST, ALT and ALP level in the

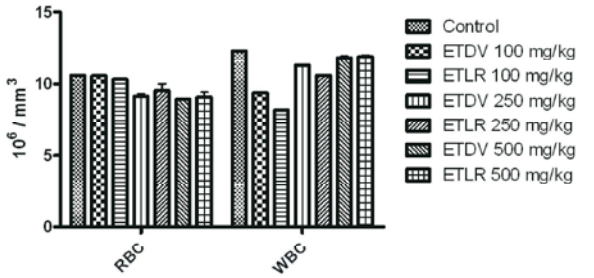

Fig. 6: Effect of ETDV and ETLR on Haematological (RBC and WBC)

treated rats at higher dose of 500 mg/kg as compared with the control rats.

Effect of ETDV and ETLR on Haematological Parmeters in Rats: From the study it was evident that there was a significant increase ($P < 0.05$) in the haemoglobin contents, RBC and WBC count with the higher dose (500 mg/kg) treated rats when compared with control rats. The results are shown in figure 5, 6 and table 6, where group I animals were treated with Tween 80 (5 ml/kg), group II and III animals with 100 mg/kg of ETDV and ETLR, group IV and V animals with 250 mg/kg of ETDV and ETLR, group VI and VII animals with 500 mg/kg of ETDV and ETLR.

DISCUSSION

The evaluation of sub-chronic and chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the plant preparation. The highest overall concordance of toxicity in animals in comparison with humans is with haematological, gastrointestinal and cardiovascular adverse effects, while certain adverse effects in human's especially hypersensitivity and idiosyncratic reactions, are poorly correlated with toxicity observed in animals [15].

In the present study, where the acute toxicity study of ETDV and ETLR was carried out as per OECD-423 guidelines, no mortality was observed in both the animals of the control group as well as animals treated with a maximum dose of 2000 mg/kg. Hence $1/10^{th}$ of 2000 mg/kg i.e. 200 mg/kg of dose was selected as a maximum dose for sub-acute toxicity study [16].

The results of sub-acute toxicity study showed that there was no significant change in animal behaviour due to the absence of toxicity. The animals treated with ETDV and ETLR showed normal growth pattern and body weight compared with control rats and when treated with Tween 80. So the changes in body weight can be used as an indicator of adverse effects of drugs and chemicals [17-19].

The changes in enzymes like ALP, AST and ALT levels showed liver impairment, due to toxicity [20]. Serum cholesterol mainly regulated via synthesis in the liver and increase or decreases in serum concentrations of constituents suggest liver toxicity. The results of the present study were assessed after 28 days of administration of ETDV and ETLR and it was found the ETDV and ETLR at all concentration did not produce liver damage.

There was a slight decrease in plasma glucose level when higher doses of ETDV and ETLR (500 mg/kg) were administered in the treated rats. There is need to further study to confirm the hypoglycemic activity of leaves of DV and LR.

Analysis of blood parameters is risk evaluation as the change in haematological system has a higher predictive value for human toxicity, when data are translated from animal studies. After 28 days of treatment, there were no significant changes in the levels of WBC and RBC between control and test group animals following repeated administration of ETDV and ETLR. Interestingly, significant increase in the levels of haemoglobin was found in treatment with ETDV and ETLR with a higher dose of 500 mg/kg. The possible reason could be that one of the constituents of ETDV and ETLR may increase absorption of iron.

CONCLUSIONS

The overall results suggest that ETDV and ETLR are non-toxic to the haematopoietic and leucopoietic system. The haematopoietic and leucopoietic systems are the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal [21]. Therefore, it is possible to assume that the extract is non- haemotoxic. The above observation establishes the non-toxicity of ETDV and ETLR at a concentration of 2000 mg/kg. Based on these results, further studies on animals with ETDV and ETLR will be carried out.

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