BIO-ASSAY OF TINCTURE DIGITALIS[1]

By

R.P. PATEL AND V.B. DESAI

From the department of Pharmacology, L.M. College of Pharmacy, Ahmedabad

(Received March 31, 1962)

A simple method of standardisation of tincture digitalis on male frogs, *Rana tigrina* by slow intravenous infusion into caudal end of the anterior abdominal vein has been suggested. The results show that quantitative estimation can be done by this method.

As there is no satisfactory chemical method of assay for digitalis, it is standardised biologically. Various methods of bio-assay of digitalis have therefore been proposed by various workers (De Lindvan, 1926 ; Erik Knaffl-enz, 1926, Burn 1950) using different animals such as cats, guinea-pigs, frogs etc. The methods using frogs are very convenient and economical. These are based on the percentage mortality responses in certain groups of frogs, when given in graded doses into the lymph sac. Bhatia (1934) has studied this lymph sac method on *Rana tigrina* and described a characteristic curve. Pharmacopoea of India (1955) suggests a method using 80 frogs, *Rana temporaria* weighing between 15 to 30 g but these frogs are not available in our country. Ghosh *et al.* (1958) selected *Rana tigrina,* weighing between 25 to 30 g for the lymph sac method and got satisfactory results, but this requires 80 frogs and moreover, it is difficult to get even *Rana tigrina* weighing between 25 to 30 g.

Trevan *et al.* (1928) has studied intravenous infusion of diluted tincture of digitalis on *Rana temporaria* frog. These frogs are not available in India but *Rana tigrina* frogs weighing 100 to 250 g are very common in India. It was therefore, thought interesting to study the effect of tincture digitalis by slow intravenous infusion in the anterior abdominal vein with a view to its standardisation on this species of frogs. Our results have shown that quantitative estimation can be done, using twelve male frogs weighing between 100 to 250 g.

METHODS AND RESULTS

Male *Rana tigrina* frogs were selected for the experiment. To avoid the possibility of variation amongst frogs, it was thought proper to divide the

1 Part of the thesis by Mr. V.B. Desai is in partial fulfilment of the M. Pharm. Degree of the Gujarat University.

frogs arbitarily into three groups, *viz*, frogs weighing between 100 to 150 g, 150 to 200 g, 200 to 250 g and to study the effect of tincture digitalis known and unknown on each group. A tincture digitalis B.P. was diluted to 10 times and 15 times with 0.6 per cent w/v sodium chloride, in distilled water. These two known dilutions were used for a preliminary study Six frogs were employed for studying each dilution in each weight group. Precaution was taken to see that the distribution of the weight of individual frog for one dilution, in a particular weight group was approximately the same as those employed for other dilution. The frogs were kept in fresh water for three days, before they were taken for study. Each frog was pithed and the caudal end of the anterior abdominal vein was dissected and then cannulated. The cannula was then connected to a standard glass burette. The diluted tincture was then allowed to run with a steady rate of 0.25 ml per minute from the burette, until the heart stopped in systole. This volume was recorded as a minimal lethal dose for each frog. In a similar manner, individual minimal lethal doses of all the frogs were determined for each dilution. These results are tabulated below :—

Group of frogs weighing between	Mean log M.L.D./100g body wt. 1 : 10 diluted tincture (A)	Mean log M.L.D./100 g body wt. 1 : 15 diluted tincture (B)	Ratio of potency A/B	% deviation from known value. $\frac{A/B-1.5}{1.5}$ 100	S.E.[1] of the mean of difference	Fiducial limits of R.P. at p 0.99 df-10
100-150 g	0.6396	0.7862	1.402	6.533	0.0192	86.62 to 115.0 %
150-200 g	0.6997	0.8485	1.409	6.067	0.0275	81.75 to 122.3 %
200-250 g	0.7053	0.8627	1.436	4.267	0.0305	80.04 to 124.9 %

1 S.E. standard error. df-degree of freedom.

The two dilutions studied had a relative potency of 1.5. The experimental data also shows that (i) the relative potency for all weight groups does not deviate by more than 6.533 per cent of the known value and (ii) the limits of error are also within the Indian Pharmacopoeal limits (at p 0.99, 40-170 per cent). Moreover, it may be seen from the table that the limits of error are least for the groups 100 to 150 g. It was therefore, decided to carry out the standardisation of tincture digitalis on this particular weight group. For this purpose two tincture digitalis I.P. were prepared from inter-

national digitalis leaf powder, by the Indian Pharmacopoeal maceration process These were labelled as A and B. These were then diluted to 10 times with 0.6 per cent w/v sodium chloride in distilled water and studied on male frogs weighing between 100 to 150 g. Six frogs were used for each tincture. From the volume of the diluted tincture required just to stop the heart, lethal dose of each diluted tincture for each frog was determined and its log dose 100 g of body weight calculated.

The log of the minimal lethal dose of the diluted tinctures (A and B) per 100 g of the body weight of the frog is given in the following table :—

A			B		
Wt. of frog (g)	Lethal dose (ml)	Log dose/ 100 g body wt	Wt. of frog (g)	Lethal dose (ml)	Log dose/ 100 g body wt
107	3.7	0.5388	103	3.4	0.5187
125	4.8	0.5843	120	5.4	0.6532
113	4.1	0.5597	113	3.6	0.5032
140	4.6	0.5167	149	5.7	0.5827
110	4.6	0.6214	108	3.9	0.5577
103	3.8	0.5670	102	3.8	0.5712
Mean = (m1) = 0.5647			Mean = (m2) = 0.5645		

Mean of the difference M = m1 − m2 = 0.0002. Ratio of potency is anti-log. 0.0002 − 1.005 i.e. 100.5% standard error of the mean of the difference S.E. − 0.0264. Fiducial limit at P = 0.99 is 82.43 − 121.4% (d.f. = 10).

DISCUSSION

The results show that the ratio of potency is 100 5 per cent, while I. P. limits lie between 90-110 per cent. The fiducial limits at P = 0.99 is 82.43 − 121.4 per cent which is also within the I.P. limits. Only twelve male frogs are required for the complete bio-assay, instead of 80 by the lymph sac method. Moreover, this method is simple to operate and the time consumed is about 4 to 5 hrs. This method may, therefore, be used for the standardisation of digitalis preparations using male *R. tingrina* which are freely available in India.

REFERENCES

De Lindvan Wijin Gaarden (1926). *Arch. Exp. Path. Pharmk.*, **113**, 40.

Erik, Knafflenz. (1926). *J. Pharmacol.*, **29**, 407-25.

Burn, J.H. (1950). 'Biological Standardisation', IInd ed., P. 295, Oxford Medical Publications.

Bhatia, B.B, (1934). *Ind. J. Med. Res.*, **22**, 3.

Pharmacopoeia of India, (1955). 1st ed., p. 938-939 Govt. of India Press, Calcutta.

Ghosh, A.K.. Srivastava, R.K. and Tayal, J.N. (1958). *J. Pharm. Pharmacol.*, **10**, 175.

Trevan, J.W. and Ellen, M. Boock (1928). Quart. J. of Pharm., **1**, 13.