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Toxicological study of the different organs of *Corchorus olitorius* L. plant with special reference to their cardiac glycosides content

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With 5 tables

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Corchorus olitorius L. plant is a common edible vegetable, the leaves of which are the material of a very popular Egyptian dish (Mulukhia). It belongs to the family *Tiliaceae* (3). It has long been known to produce some toxic manifestation, and in this respect the seeds are reported to be highly toxic due to their cardiac glycosides content (4, 9, 10, 11).

As this plant is widely cultivated in Egypt and is used by animals and for human consumption, its toxicological study seemed essential to determine the correlation between the toxicity and the cardiac glycosides make-up of the different parts of the mature edible plant.

Material and Methods

1. The alcoholic extracts

500 g of the mature, dry powdered plant material (seeds, roots, stems and leaves) were directly defatted with petroleum ether (60-80 °C), then exhaustively extracted with 70% ethanol by percolation at room temperature. The percent of the residue of each extract was obtained upon concentration of the corresponding extract under vacuum and was calculated on dry weight base of each organ as shown in table 1.

2. Estimation of the cardiac glycosides content of the tested extracts

The percent of the cardiac glycosides in the alcoholic extracts of the different plant organs was estimated colorimetrically as strophanthidine according to the method described by Negm et al. (7) and the results are cited in table 2.

3. Determination of LD₅₀ of the alcoholic extracts of the different plant organs

The LD₅₀ of the solutions of the dried alcoholic extracts of the different tested plant organs in distilled water was determined in mice by intraperitoneal injection. Four hundred mice were picked randomly from the animal house colony of the N.R.C., divided into groups of 10 animals each, weighing from 20 to 30 g, of both sexes. Preliminary experiment was carried out for each extract to find out the range of lethal and non-lethal doses. This was followed out by a large-scale experiment to demonstrate the dose-lethality relationship for each extract. After injection, the animals were supplied with the usual laboratory diet and water and were observed for any possible toxicological changes. Mortality percentage was recorded during a 24-hours period in each dose level and post-mortem examination was performed.

Table 1. Percentage of the total alcoholic residues of the different organs of *Corchorus olitorius* L. plant.

Plant organs	Total alcoholic residue ½
Seeds	24
Roots	27.21
Stems	28.04
Leaves	31.5

Table 2. Percentage of cardiac steroid fraction in the alcoholic extracts of the different organs of *Corchorus olitorius* L. plant.

Plant organs	% of cardiac steroids
Seeds	0.412
Roots	0.011
Stems	0.023
Leaves	0.002

The results were assessed statistically for the determination of the LD₅₀ according to Miller Tainter method as shown in table 3.

Two groups of 5 mice each of about the same weight and sex were used. The first group was injected intraperitoneally by a dose equal 1/10 of the LD₅₀ dose of the alcoholic extract of the seeds for 60 days. The other group was kept under the same condition as the control. The animals were individually marked. Symptoms were noticed during the course of injection and haematological examination was done after 60 days for both groups.

Blood samples were taken from animals by cardiac puncture as described by Lane Petter et al. (5). Determination of haemoglobin concentration, enumeration of erythrocytes and leucocytes and determination of packed cell volume were performed according to the method of Schalm (8). The blood sedimentation rate was determined by Landau's method (5) modified in that the blood sample with the anticoagulant was directly used and the pipettes were kept at 37 °C. Readings were taken in millimeter after one and two hours. Mean corpuscular volumes, corpuscular haemoglobin and mean corpuscular haemoglobin concentrations were calculated as indicated by Schalm (8). The results were analysed statistically using standard error and analysis of variance as demonstrated in tables 4 and 5.

Table 3. The LD₅₀ of the solutions of the alcoholic extracts of the different organs of *Corchorus olitorius* L. plant.

Plant organ	LD ₅₀ in mg/kg body weight
Seeds	895.652 < 922 < 928.348
Roots	2544.0 < 2570 < 2595.944
Stems	2263.22 < 2266 < 2268.78
Leaves	5761.56 < 5821 < 5880.44

LFL¹ < LD₅₀ < UFL²

1 = Lower fiducial limit

2 = Upper fiducial limit

Table 4. Effect of the alcoholic extract of *Corchorus olitorius* L. seeds on blood picture of mice.

Blood constituents	Control	Test
Red blood corpuscles	7.731 \pm 2.08	10.792 \pm 2.08
White blood corpuscles	4.660 \pm 0.756	3.040 \pm 0.756
Haemoglobin	13.0 \pm 0.97	13.7 \pm 0.97
Packed cell volume	37.0 \pm 1.03	38.4 \pm 1.03
Blood sedimentation rate	0 \pm 0	0 \pm 0
Mean corpuscular volume	0.000039 \pm 0.0061	0.00004 \pm 0.0061
Mean corpuscular haemoglobin	0.0000137 \pm 0.00063	0.0000127 \pm 0.00063
Mean corpuscular haemoglobin concentration	35.18 \pm 1.37	35.09 \pm 1.37
Leucocytic count		
Lymphocytes	60.2 \pm 5.65	60.0 \pm 5.65
Eosinophiles	0 \pm 0	0 \pm 0
Neutrophiles	38.4 \pm 5.75	37.2 \pm 5.75
Monocytes	1.4 \pm 0.77	2.8 \pm 0.77
Basophiles	0 \pm 0	0 \pm 0

Values are means of five determinations \pm SE.

Results and discussion

The LD₅₀ and its fiducial limits of the solutions of the alcoholic extracts of the seeds, roots, stems and leaves of *Corchorus olitorius* L. plant were determined statistically as shown in table 3. The acute toxic symptoms exhibited by all the tested extracts were generally hurried respiration, diarrhoea and death. Congestion of the heart and other internal organs was observed in the P.M. of all the tested preparations.

The chronic toxicity of the alcoholic extract of the seeds revealed no significant difference in blood picture in mice injected for a period of 60 days with $\frac{1}{10}$ from the LD₅₀ dose of this extract as demonstrated in table 4 and 5.

The chronic toxic symptoms observed were depression and emaciation, losing weight, mucous membrane of the eyes was cyanosed and the hair was easily detached. The pathological legions were mainly general venous congestion, flabby heart muscle and enlargement of liver, kidneys and spleen.

From the above results, it was found that the different plant organs contain cardiac steroids but in variable amounts. It is about 0.412% in the seeds, 0.011% in the roots, 0.023% in stems and 0.002 in the leaves as shown in table 2. This result declares the variation of the potency of the toxicity of the different organs expressed in term of the LD₅₀ of each extract. In this respect, the seed extract was the most toxic preparation as it contains the highest percent of cardiac steroids, while the leaves, stems and roots come next as shown from tables 2 and 3. These results were in agreement with that obtained by *Beiova* and *Turova* (1) who compared the toxicity of different concentration of cardiac glycosides of strophanthine-like activity and found that different glycosides and concentrations of them reflect different degree of harmfulness for the body.

Table 5. Analysis of variance of the effect of the alcoholic extract of seeds on blood picture of mice.

Blood constituents	S.V.	D.F.	S.S.	M.S.	F
Red blood corpuscles	Total	9	146.572		
	Test	1	23.430	23.43	1.826
	Error	8	173.142	21.543	
White blood corpuscles	Total	9	29.449		
	Test	1	6.561	6.561	2.2937
	Error	0	22.884	2.805	
Haemoglobin	Total	9	4.025		
	Test	1	1.225	1.225	2.578
	Error	0	43.800		
Packed cell volume	Total	9	48.1		
	Test	1	4.9	4.9	0.9074
	Error	8	43.2	5.4	
Mean corpuscular volume	Total	9	16990.4		
	Test	1	2073.6	2073.6	1.1121
	Error	8	14916	1864.6	
Mean corpuscular haemoglobin	Total	9	1852.80		
	Test	1	230.40	230.40	1.1366
	Error	8	1621.60	22.7	
Mean corpuscular haemoglobin concentration	Total	9	77.1918		
	Test	1	1.2724	1.2724	0.1341
	Error	8	73.9144	9.4899	
Lymphocytes	Total	9	1278.9		
	Test	1	0	0	0
	Error	8	1278.9	0	
Neutrophils	Total	9	1326.8		
	Test	1	3.6	3.6	0.0217
	Error	8	1326.0	165.75	
Monocytes	Total	9	28.9		
	Test	1	4.9	4.9	163
	Error	8	24.0	3.0	

S.V. = source of variance; D.F. = degree of freedom; S.S. = sum of square; M.S. = mean square

The chronic toxicity investigation of the alcoholic extract of the seeds (most potent extract) had shown that the blood picture in mice injected for a period of 60 days with this extract was not affected. However, external and internal toxic symptoms were observed. This may be due to the toxicity exhibited by the accumulation of cardiac glycosides residue in this source as previously reported (4, 9, 10, 11).

Further work for the determination of the chronic toxicity of the leaves by feeding to rats for 6 months is still in hand to demonstrate extents of toxicity of this edible part though it contains the least amount of the toxic active principle as shown from the present investigation.

Summary

The acute toxicity of the alcoholic extracts of seeds, roots stems and leaves of the fully mature *Corchorus olitorius* L. plant was determined in mice by intraperitoneal injection. The cardiac glycosides content of each extract was estimated and the correlation between the two investigated parameters was established. The chronic toxicity of the alcoholic extract of the seeds was determined in term of its haematological and symptomatic effects on mice upon intraperitoneal injection for a period of two months.

References

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