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Abortifacient and toxic actions of the glycoside 'albitocin' extracted from some *Albizia* species

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Conscious intact mice, rats, guinea-pigs, rabbits and monkeys were dosed with "albitocin", an active glycoside extracted from plants of certain *Albizia* species used by East African native doctors to accelerate labour and procure abortion. In pregnant animals abortion usually occurred within 12 hr at dose levels characteristic for each species irrespective of stage of gestation. In larger doses the drug was toxic, and with lethal doses animals survived 12–170 hr, with increasing apathy and anorexia, conscious but moribund as death approached. Toxicity in the orally dosed animals was lower than in those dosed intraperiotoneally or intravenously. The changes observed which could account for the mortality are discussed.

THE isolation of an oxytocic glycoside, albitocin, in the form of an amorphous white powder, from some members of the plant genus *Albizia*, its partial chemical characterization (Lipton, 1959, 1960, 1963), its actions on uterine strips *in vitro* (Lipton, 1963), and its actions on the blood pressure and uterus of anaesthetized mammals (Lipton, 1964) have been described previously. Some of its actions on conscious intact mammals, with details of attempts to determine the nature of its toxicity are now described.

Experimental

Mice, rats, guinea-pigs, rabbits and vervet monkeys (*Cercopithecus aethiops*) were dosed by one of four routes: gastric, via a semi-stiff polythene tube of 2 mm external diameter, lubricated with glycerol; intraperitoneal (ventral abdomen); intravenous (ear vein in rabbits, short saphenous vein in monkeys); intramuscular (semitendinosus muscle in guinea-pigs).

All doses and injections were given in 0·9% saline solution with full sterile precautions in conscious immobilized animals and were controlled by administration of equivalent volumes of the saline solution to comparable animals in each series.

As in previous work, the assumption has been made that albitocin is the only new active principle present in the plant extracts. Potency was determined by assay on the gravid guinea-pig uterus *in vitro* with appropriate controls. Animals were fed *ad lib.* on suitable green food and balanced diet pellets enriched with vitamins. They were marked and weighed daily, some had rectal temperature recorded, and those that died or were killed after dosing were examined post mortem for gross or microscopic changes.

The gravid state was detected by palpation, confirmed where necessary by X-ray photography, and abortion was only recorded if conception products were seen in the cage, or if a significant fall in weight occurred overnight and the uterus was clearly post-partum when the animal died or

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was killed. (In such instances foetuses, etc. were assumed to have been eaten.) The aborted foetus weights and crown-to-rump lengths were recorded where possible.

HAEMATOLOGY

Serial blood samples were taken from 8 rabbits before and at 6 hr intervals after lethal doses of albitocin, continuing to the time of death; immediately after the heart stopped a further sample was taken direct from the ventricles by thoracic puncture. Standard techniques were used with the blood samples from some of the rabbits for haemoglobin, haematocrit, specific gravity, erythrocyte count, and plasma protein, glucose, sodium and potassium concentrations, alkali reserve and pH.

Blood bacteriology. Samples were: incubated under hydrogen by the method of Mackie & McCartney (1953); cultured on blood agar, McConkey's bile-salt agar and incubated for 24 hr; examined microscopically as a smear stained with Leishman and Giemsa stains.

Results

GASTRIC TUBE, INTRAVENOUS AND INTRAPERITONEAL ADMINISTRATION

The ED50 (abortion) and LD50 doses by these routes were calculated by the method of Litchfield & Wilcoxon (1949) and are summarized in Table 1. Only approximations were possible for the rabbits and monkeys owing to the relatively short series available. No data were obtained for abortifacient activity by albitocin in very early gestation, but above a foetal crown-rump length of 0·5 cm abortions were produced at all stages of gestation. The crown-rump (C–R) length of aborted mice foetuses ranged from 0·6–2·3 cm; aborted rat foetuses ranged from 1·0 cm C–R length, wt 0·48 g up to 5·0 cm C–R length, wt 5·8 g; guinea-pig foetuses ranged from 0·8 cm C–R length, wt 0·45 g to 10 cm C–R length, wt 70 g.

TABLE 1. EFFECTIVE DOSES FOR ABORTION (ED50) AND LETHAL DOSE (LD50) IN VARIOUS SPECIES AFTER ALBITOCIN

Species	No.	Route	ED50 mg/kg	LD50 mg/kg
Mouse ..	355 120	i.p. i.v.	3·2 ± 0·6 —	5·9 ± 0·6 6·0 ± 0·3
Rat ..	54	i.p.	0·5 ± 0·1	0·8 ± 0·1
Guinea-pig ..	75 84	Gastric i.p.	11·0 ± 2·5 0·7 ± 0·2	19·0 ± 5·0 1·0 ± 0·2
Rabbit ..	42	i.v.	1·0 ± 0·3	1·8 ± 0·5
Monkey ..	17	i.v.	Approx. 1·0	Approx. 2·5

Aborted foetuses observed soon enough after expulsion were alive and normal in appearance and movements. Several guinea-pig foetuses aborted near term, even by females given lethal dosage, were sufficiently mature to survive with foster mothers and showed no subsequent abnormalities. It was concluded that the drug either possessed low foetal toxicity or did not traverse the placenta.

Plant extracts used by native doctors are taken by mouth, so the abortifacient and lethal dosage was also determined by this route in guinea-pigs. Results (Table 1) indicate that both abortifacient action and toxicity were much less in these animals, supporting earlier observations that gastric tube administration of up to 5·0 mg/kg albitocin failed to increase contractions in uteri of anaesthetized gravid guinea-pigs (Lipton, 1964).

INTRAMUSCULAR ADMINISTRATION

Seventeen gravid, 18 non-gravid female and 16 adult male guinea-pigs were injected with single doses in the range 0·6–2·0 mg/kg albitocin in one semitendinosus muscle. Saline-dosed control animals showed no effects. Within a few hr, the injected muscle in all the dosed animals was swollen and hard, and the leg was dragged, with no attempt to use it. All reflexes were present, but weak where the injected muscle was involved. Direct stimulation with needle electrodes showed that all the muscles in the limb distal to that injected were normal when stimulated directly or via the motor nerves. The injected muscle was sluggish and weak in response to such stimulation, but still capable of contracting. Squeezing or stretching it did not appear to be painful to the animals. The body weights were maintained, the animals were otherwise normally active and no abortions or deaths occurred. From this absence of systemic effects it was concluded that the drug injected intramuscularly did not escape, or escaped only slowly into the general circulation.

After 2–4 weeks the swelling subsided and the animals began to use the limbs again, and by 6 weeks after the injection appeared normal.

NATURE OF TOXICITY

Mice dosed intraperitoneally with 8·0 mg/kg, rabbits intravenously with 3·5–7·0 mg/kg and monkeys intravenously with 4·0 mg/kg were examined for effects of the drug during survival and immediately after death and in some cases after killing at intervals following the dose.

These animals and all others receiving lethal dosage showed similar symptoms, a gradual development of anorexia, and a concomitant decline in weight, a sleepy appearance and apathy to handling. There was no diuresis but some showed signs of diarrhoea. Respiration sometimes slowed slightly and changed to deep gasping during the last 30 min before death. Rectal temperature was constant.

Examination of the blood samples from the rabbits showed small falls in haemoglobin and haematocrit values of comparable size in both dosed and control animals, proportional to the volume of the sample removed. No haemolysis occurred at these dose levels. Specific gravity was constant at 1·055 throughout and pH was unchanged at 7·4. The alkali reserve fell significantly more in the dosed than in the controls (from 21 to 13 m-equiv./litre serum HCO_3^-) then rose before death to 18 m-equiv./litre. Plasma albumin fell significantly from 3·4 $\pm$ 0·2 g/100 g plasma to 2·8 $\pm$ 0·2 g/100 g but in some animals this was partly restored before death.

No consistent change in plasma sodium concentration occurred but plasma potassium concentration rose from 5·0 ± 0·3 m-equiv./litre to 8 ± 1·2 m-equiv./litre. One animal, however, showed no change in plasma potassium concentration up to the time of death.

On the first day the dosed animals' blood glucose showed a rise from 130 ± 15 to 205 ± 11 mg/100 ml which did not occur in the controls, but this fell to 185 ± 16 mg/100 ml before death.

Erythrocytes showed no change in shape or size or sedimentation rate (6·5 ± 2·5 mm/min). Leucocyte count rose from 4200 ± 480 to 5400 ± 650/mm^3. Control leucocyte counts remained constant.

At no time was there any sign of an infective process to account for death in the dosed animals; cultures of the blood, intraperitoneal fluid and nasal mucus showed only normal flora. Common ecto- and endo-parasites were present, but the only pathogens detected in the blood were microfilaria, which were present in similar numbers in the control animals.

POST-MORTEM AND HISTOLOGICAL EXAMINATIONS

Apart from the increased leucocyte count referred to above, the blood picture remained unchanged up to death. Smears of the intraperitoneal fluid also showed no abnormalities subsequent to dosing by any route.

None of the earlier studies showed abnormality in frozen or paraffin wax sections of the heart, lungs, liver, kidneys, skeletal muscle, brain, intestinal and gastric mucosae, adrenal glands, bladder, ovaries and testes using standard staining techniques.

When this work was repeated later, however, necroses were observed in the renal tubules in rabbits dosed with a total of 8 mg/kg albitocin, and Kerr & Pound (1966) reported liver necroses in mice and rats dosed with albitocin (see Discussion).

Discussion

ABORTIFACIENT ACTION OF ALBITOCIN

In addition to its spasmogenic action on the isolated uterus and the uteri of anaesthetized animals *in situ*, albitocin in adequate dosage produced a high abortion rate in intact mammals of all species tried at any stage of gestation. This gives strong support to the use of the plant by African witch-doctors for acceleration of labour and abortion. There are superstitious reasons for a desire for rapid parturition among women of African Bantu tribes beyond the normal wish to reduce pain and these are discussed elsewhere in detail (Lipton, 1960). Powerful uterine spasmodics are known and are used in most births, often even where these take place in modern hospitals.

It is probable that the exceptionally high incidence of uterine rupture in Uganda (Rendle-Short, 1960) is due in part to this practice. Where such spasmodics are taken in large doses or too early in labour, especially if disproportion is present or there is any other obstruction to normal birth, a high incidence of uterine rupture is not surprising.

THE TOXICITY OF THE ACTIVE PRINCIPLE

The cause of death in those animals overdosed with albitocin has not yet been determined in all the species but evidence of necrosis in renal tubules was observed in rabbits, and Kerr & Pound (1966) showed that albitocin intraperitoneally produced an elaborate pattern of necrosis in the livers of mice and rats well correlated with the outward symptoms of toxicity and death, commencing with enlargement of parenchymal cell nuclei and peripheral zone fatty infiltration and progressing to coagulative necrosis in the intermediate zone, with eventual irreversible cytoplasmic and nuclear damage in the outer two-thirds of the lobules. These effects were not modified by dietary enrichment with tocopherol or by administration of promethazine hydrochloride. Oral dosing produced much less necrosis. Kerr later observed some centrilobular necrosis in mice about 14 days pregnant, after similar dosage (Dr. J. F. R. Kerr, personal communication). There is no information on the means by which the necrosis is produced by the drug, nor on how subsequent symptoms leading to death are produced, but these do fit a hepatic-coma type death.

Changes in plasma sodium were slight and variable and increase in plasma potassium concentration suggests increase in cell membrane permeability, but the electrical activity of the heart did not show any consistent changes (Lipton, 1963), and death from the drug was not rapid even when massive doses were given, but always took at least 12 hr, sometimes up to 7 days.

Many dosed pregnant animals aborted the uterine contents and died subsequently, but, given suitable dosage, many aborted and survived for the normal life span. This suggests mild or reversible actions of the drug in these instances. It also raises the possibility that, in addition to the previously reported uterotonic property, actions on the liver might also be involved in the abortifacient action.

It is possible that the human uterus *in situ* is more sensitive to the drug than that of lower animals, and that oral doses well below the toxic level are effective in accelerating labour. This seems likely if albitocin is the effective constituent of the native medicine.

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