

Histological Studies of the Gastro-Intestinal Tract of the Rat After Poisoning With Tubers of *Gloriosa Superba*

by

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Poisoning due to ingestion of *Gloriosa superba* is not uncommon in Ceylon. This may happen accidentally as these tubers resemble sweet potatoes. The tubers may also be taken for suicidal purposes.

The poisonous effects of *Gloriosa superba* have been known for a long time. The toxic agent was first identified by Clewer, Green and Tutin in 1915. Working with dried tubers they isolated a mixture of alkaloids consisting chiefly of colchicine together with small amounts of two other unidentified crystalline bases. They concluded that the toxic properties of the tubers were essentially due to colchicine.

The first pharmacological study of colchicine was done by Jacobi in 1890 (quoted by Dixon and Malden, 1909). Jacobi showed that after the injection of colchicine into an animal, symptoms of poisoning did not appear for 2 to 3 hours after the injection. He concluded that colchicine excited some portions of the nervous mechanisms of the stomach and intestines causing vomiting and diarrhoea and that death was ultimately due to paralysis of the respiratory centre.

Dixon and Malden (1909) divided the actions of colchicine into immediate and remote effects. The immediate effects were the augmentation of the automatic movements of smooth muscles throughout the body resulting in increased intestinal peristalsis and increased tone of uterine, splenic and bronchiolar muscle. The remote effects were a gradual depression of the central nervous system and changes in blood consisting of a transient leucopenia followed by leucocytosis.

Jacobson in 1925 (quoted by Manske and Holmes, 1925) studied the actions of colchicine on the frog's heart, and the uterus and intestines of the rabbit. He showed that colchicine had an inhibitory effect on those organs, an observation contrary to those of Dixon and Malden (1909).

The fate of colchicine injected into an animal was studied by Brues (1942). After a single intravenous injection of colchicine into a rat, there was a rapid fall in blood concentration followed by stabilisation at a low level after a few minutes. The tissues contained less of the alkaloid than blood. Elimination was through the bile and the intestines. Within a few hours, 10 to 25 per cent of the injected dose was found in the intestinal wall and its contents. Elimination by the urine lasted for a short time when the blood concentration was at its highest. No urinary elimination of the drug took place when the blood level was low. Within 16 hours, 50 per cent of the drug had been eliminated. There was no evidence for its transformation into a more toxic substance nor for any selective tissue fixation.

Colchicine is an antimetabolic drug (Goodman and Gilman, 1965). Leblond and Stevens (1948) used colchicine as an antimetabolic poison to estimate the renewal of the intestinal epithelium in the rat. In a study of colchicine on normal and neoplastic tissues in mice, Clearkin (1937) reported visible effects in the cells of jejunal crypts within 4 hours of an injection.

The general pharmacology of colchicine has been described by Ferguson (1952) who showed that the most prominent action of colchicine was on the central nervous system and that the acute physiological disturbances were reflections of this effect.

METHOD AND MATERIALS

Two sets of experiments were carried out using adult hooded rats of both sexes. In one experiment, watery extract of tubers of *Gloriosa superba* or colchicine solution was given orally. In the other, colchicine solution was injected intraperitoneally.

First Experiment. Ten rats were given a single oral dose of watery extract of the tubers while 10 others were given a single oral dose of colchicine solution. The doses given were watery extract equivalent to 10 G fresh tuber per Kg body weight and colchicine 5 mg per Kg body weight. A pilot study indicated these amounts to be an approximate LD 50. The concentrations were adjusted so that the required dose was present in 2 ml of the extract or colchicine solution. Ten control animals were each given 2 ml distilled water orally. All 20 rats given the watery extract of tubers or colchicine solution developed diarrhoea between 6 and 8 hours after administration. The controls did not develop diarrhoea. Of the rats that had diarrhoea, 4 given colchicine and 4 given watery extract of tubers were killed for histology, 48 hours after administration of the drug. Of the remaining 12 rats, two rats given the watery extract and three given colchicine solution died on the 3rd day. 7 rats survived.

Four control animals were also killed for histological study.

Second experiment. Twenty rats were given a solution of colchicine 10 mg per Kg body weight in 0.5 ml intraperitoneally. Twelve rats in this group developed diarrhoea after 2 hours. All died between 5 and 8 hours after injection. Control animals were injected with 0.5 ml distilled water intraperitoneally.

Portions of the gastrointestinal tract of rats that developed diarrhoea in both experiments as well as in control animals were taken for histological study by the following method:

The rat was stunned by a blow on the head. The abdomen and thorax were opened. A fine polythene catheter was tied into the descending aorta, the portal vein opened and the vascular system perfused with normal saline at a constant pressure to wash out the blood. After perfusion, 5 ml of India ink was injected through the polythene catheter. Portions of the glandular parts of the stomach, duodenum, jejunum (4" from pylorus), ileum (4" proximal to ileo-caecal junction), caecum and colon (2" from ileo-caecal) junction were taken and fixed immediately in Susa's fixative for staining with haematoxylin and eosin.

Portions of the liver, heart and kidney were also taken for microscopic examination.

RESULTS

Macroscopic

First experiment. Postmortem changes were the same in those that received colchicine solution and in those that received watery extract of the tubers. The stomachs were dilated to about 3 to 4 times the normal size and the lumina were full of watery contents. In contrast, the small intestines were contracted. On opening the small intestines, the mucosa was diffusely congested with localized areas of sloughing. At some places, especially in the ileum, the entire mucosa was seen to slough out. There were areas of haemorrhage observed both on the mucosal as well as on the peritoneal surfaces. The intestines were empty. These changes of the small intestines were not observed in the large intestines. None of the changes observed in gastro-intestinal tracts of the experimental animals were seen in the control animals.

Second experiment. The stomachs of all these rats were dilated and the small intestines contracted as in the previous experiments. Congestion, sloughing and haemorrhage of the mucosa of the small intestines were not observed in these animals.

Microscopic

First experiment. The microscopic appearance of the gut of rats given watery extract of tubers or colchicine solution orally were similar. In the glandular portion of the rat stomach, the mucosa was intact. There was no denuding of the epithelium but the mucosal capillaries showed marked dilatation. The submucosal blood vessels also showed this change (Fig. 1).

Submucosal oedema was observed. Mitotic figures were observed in the isthmus of the gastric glands but these were in keeping with mitotic figures seen in the normal histology of the rat stomach. Mitotic figures were not observed elsewhere in the gastric glands. There was no evidence of haemorrhage in the stomach wall and the muscular coat was normal.

In the duodenum and the rest of the small intestine, numerous mitotic figures were observed in the crypts of Lieberkuhn as well as in the epithelium of the villi (Fig. 2). There was generalized degeneration and necrosis of the mucosa of the small intestine. The architectural pattern of the villi was absent (Fig. 3), this change being most marked in the mucosa of the ileum (Fig. 5).

Capillary dilatation was a prominent feature of the mucous and submucous coats (Fig. 4), with oedema of the submucous layer.

There were no degenerative changes observed in the mucosa of the caecum and colon. Capillary dilatation was absent in the mucosal and submucosal layers, there was no oedema of the submucous layers and no evidence of abnormal presence of mitotic figures. The muscle coats in both small and large intestines were normal.

There were no histological changes observed in the gastro-intestinal tracts of the control animals.

Second experiment. The histology of the glandular portions of the stomachs of the rats injected with colchicine intraperitoneally showed no histological change. The mucosa and the submucosa were normal with no evidence of capillary dilatation.

In the small intestines, the mucosa and submucosa were normal and there was no denudation of the epithelium in any of the specimens examined. Capillary dilatation was not present in the mucosal and submucosal layers, and mitotic figures comparable to control animals were observed. The muscle coat was normal. The histology of the large gut was also normal. In both experiments, capillary dilatation was not observed in the histological sections of the liver, heart and kidney. A summary of results is seen in Table 1.

TABLE 1. Summary of histological appearance of gastro-intestinal tracts of rats in both experiments.

1st Experiment		Oral administration—Tuber or colchicine	
Organ	Mucosa	Submucosa	Muscle Coat
STOMACH	1. Epithelium normal 2. Mitotic figures normal 3. Capillary dilatation	1. Oedema present 2. Capillary dilatation	NORMAL
SMALL INTESTINE	1. Epithelium denuded 2. Numerous mitotic figures 3. Capillary dilatation 4. Haemorrhage	1. Oedema present 2. Capillary dilatation	NORMAL
LARGE INTESTINE	NORMAL	NORMAL	NORMAL
KIDNEY HEART LIVER	NO DESTRUCTION OF EPITHELIA OR EVIDENCE OF CAPILLARY DILATATION		

2nd Experiment		Intraperitoneal injection—Colchicine	
Organ	Mucosa	Submucosa	Muscle Coat
STOMACH	NORMAL	NORMAL	NORMAL
SMALL INTESTINES	NORMAL	NORMAL	NORMAL
LARGE INTESTINES	NORMAL	NORMAL	NORMAL
HEART KIDNEY LIVER	NO HISTOLOGICAL CHANGE		

DISCUSSION

The chief alkaloid present in the tubers of *Gloriosa superba* is colchicine which is in keeping with the fact that watery extract of tubers or colchicine produced similar clinical and histological changes in experimental rats. The degenerative changes of the mucosa were limited to the small intestines and not to the stomach and the large intestines. This is possibly due to the fact that colchicine after its entry into the entero-hepatic circulation was re-excreted *via* bile into the duodenum. Gloriosine is another alkaloid present in *Gloriosa superba* besides six others found in small quantities (Dunuwille; Balasubramaniam, and Bibile, 1966). Amongst other causes, the degenerative changes observed in the mucosa of the small intestines may possibly account for the diarrhoea produced in rats by giving *Gloriosa* tuber or colchicine orally.

When a high dose (10 mg/Kg body weight) was administered intraperitoneally, all the animals died. 60 per cent of these animals developed diarrhoea before death. The diarrhoea in this group may not be due to an intestinal cause as in the first group, since no degenerative changes were observed histologically in the gastro-intestinal tracts.

In the normal animal, cell division takes place in the crypts of Lieberkuhn and the newly formed cells migrate up the villi during their functional life. Extrusion of these cells into the lumen of the intestines occurs regularly from the tips of the villi. According to Quastler and Sherman (1959) the villus-transit time varies in different species and may even vary between different regions in the same animal. These workers also showed that the crypt cell was highly vulnerable to ionising radiations and chemical cytotoxic agents while the villus cell was resistant. Leblond and Stevens (1948) estimated that the duodenal epithelium of the normal rat was replaced every 1.6 days and that of the ileum every 1.4 days. When watery extract of tubers or colchicine was administered orally, cell division was seen to be arrested in the crypts while the villi were gradually denuded of their epithelial covering. If mitosis could be arrested for a sufficient period of time, the mucosa of the small intestines would show necrotic changes since there would be no new cells to replace the epithelial cells extruded from the tips of the villi. There is evidence, in support, to show that the ileum is more affected than the duodenum and this is probably due to the

fact that the renewal time of the epithelium in the ileum is less than that in the duodenum. The denudation of the epithelium may interfere with absorption and may also cause exudation into the intestinal lumen and aggravate salt and water loss.

The capillary dilatation in the stomach and small intestines could be either due to a localized or a generalized effect of colchicine or of the tuber. It may also have been due to inflammatory changes of the intestinal mucosa following loss of epithelium. The epithelium of the gastric mucosa was intact and the capillary dilatation in the stomach could not have been due to inflammation. Since capillary dilatation was not observed in the heart, in the liver and in the kidney, it is probable that colchicine or tuber produced capillary dilatation in the stomach and small intestines due to a localized effect.

From the results of these experiments it has not been possible to explain the cause of the dilatation of the stomach and the contraction of the intestines.

SUMMARY

Two sets of experiments were carried out on adult hooded rats of both sexes. In one set, rats were given watery extract of *Gloriosa superba* tubers or colchicine solution orally. In the other, rats were injected with colchicine solution intraperitoneally.

Rats given watery extract of tubers or colchicine solution orally showed degenerative changes in the mucosa of the small intestines. The diarrhoea in these may possibly have been due to this effect. Those injected with colchicine solution intraperitoneally did not show any histological change in the gastro-intestinal tract. The diarrhoea in this group may not have been due to an intestinal cause.

There was capillary dilatation of the mucosa and submucosa of the stomach and of the small intestines in the rats given watery extract of tubers or colchicine solution. This may have been due to a localized effect of colchicine.

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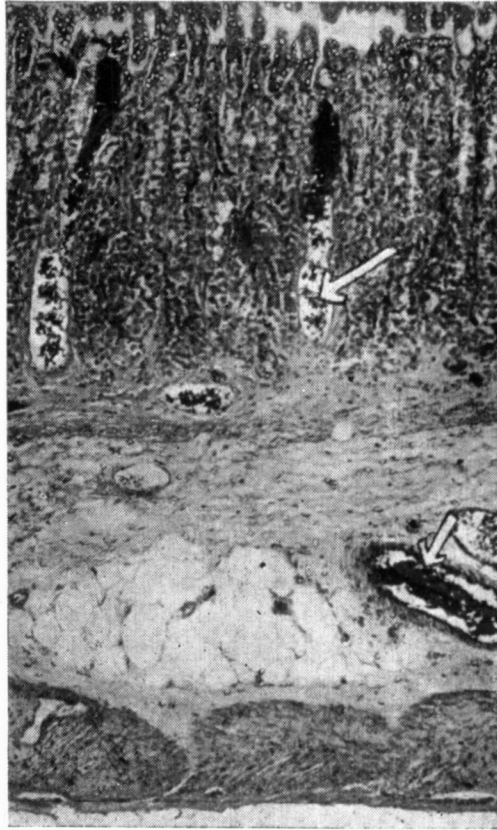


Fig. 1

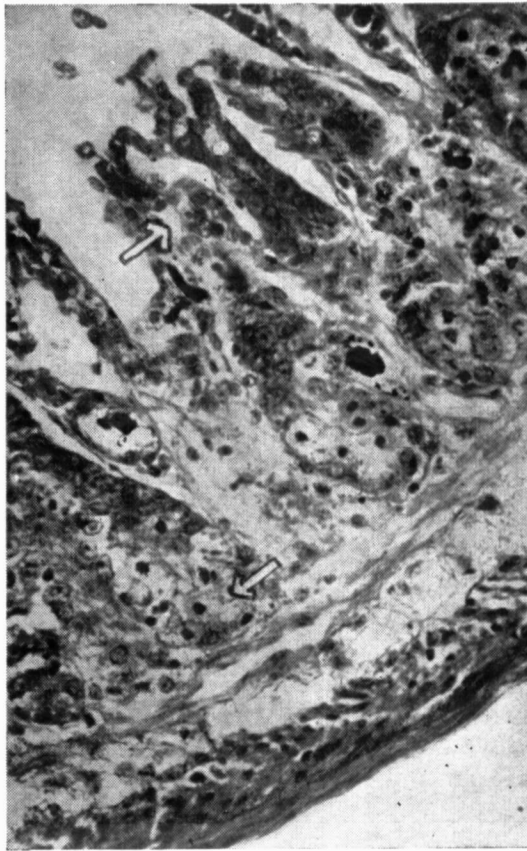


Fig. 2

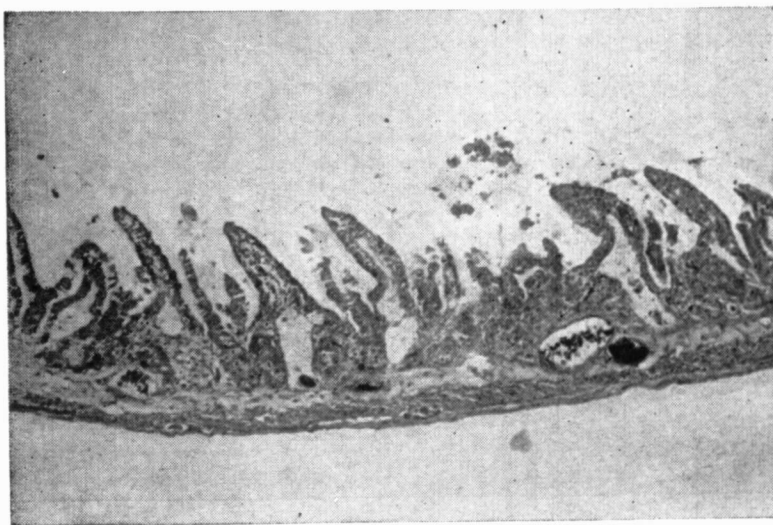


Fig. 3

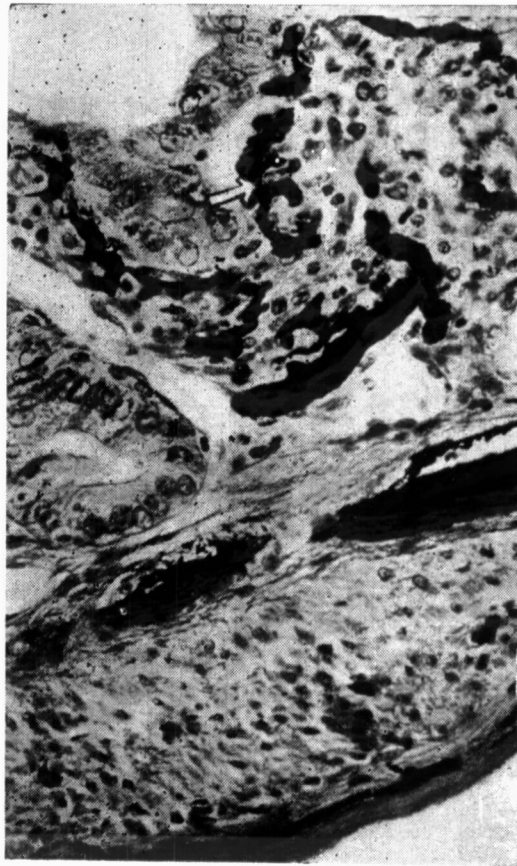


Fig. 4

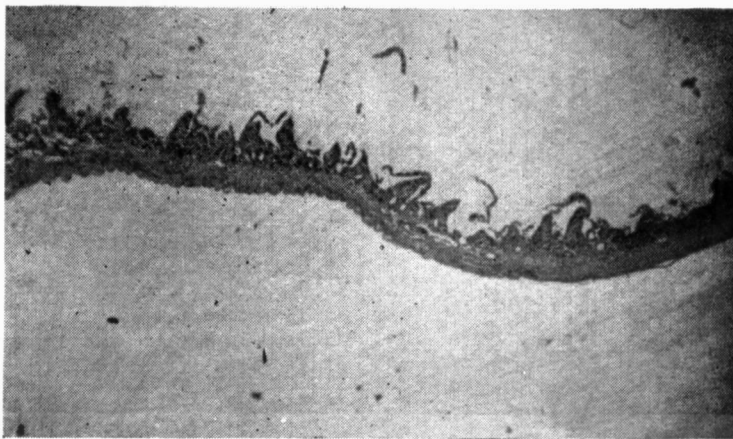


Fig. 5

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EXPLANATION OF PLATES

Plate I—

- Fig. 1. L.S. of stomach wall of rat after oral administration of watery extract of *Gloriosa superba*. Oedema of submucosa is seen. There is dilatation of capillaries (arrows) in the mucous and submucous coats. H. & E. \times 250

Plate II—

- Fig. 2. L.S. of duodenum showing mitotic figures (arrows) and shedding of epithelium of villus (top left), after *Gloriosa superba*. H & E. \times 400
- Fig. 3. L.S. of jejunum showing denuding of mucosa, after *Gloriosa superba*. H & E. \times 200.

Plate III—

- Fig. 4. L.S. of jejunum showing dilated capillaries (with India ink) in mucosa and submucosa, after *Gloriosa superba*. H & E. \times 400
- Fig. 5. L.S. of ileum showing denuded mucosa, after *Gloriosa superba*. H & E. \times 200.