SENECIO MOOREI AND ADENIA VOLKENSII TOXICOSIS IN ANIMALS

By

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S U M M A R Y

It is not possible to guess the annual loss of livestock caused by poisonous plants in East Africa but it is considerable. Investigation has also shown that some of these plants have been used for medicinal and homicidal purposes in the East African countries as well as other parts of the world.

The impetus for research on species of plants from the genera Senecio and Adenia has been the recognition of possible hepatotoxic actions as a result of ingestion of the Senecio species and the acute toxic effects caused by ingestion of Adenia species. The possibilities that dangerous toxic principles in form of alkaloids and glycosides may be contained in these plant species also needed exploration.

Cattle, pigs, sheep, rabbits and rats were used to study the pathologic effects of these plants which were obtained from various parts of Kenya. The ways of preparation of plant material for experiment were either by cutting it into small pieces; sun-drying followed by grinding into fine powder or extraction. Animals were dosed by either injections, drenching or incorporating the material in the basal diets. The toxicity in animals was evaluated by their effect on the growth rate, symptomtology, haematology, changes in serum enzymes, gross pathology and histopathology.

Pathological changes were induced in certain organs of the experimental animals by both species, namely, Senecio moorei and Adenia volkensii. In large animals, when trials were conducted for several months using Senecio moorei, the most significant lesions were observed in liver (cattle) and kidney (pigs); also present were serous effusions in the peritoneal and pleural cavities (cattle). The pathological changes in livers of cattle were portal fibrosis and slight to extensive bile duct proliferation. Varying degrees of degeneration and regeneration of hepatic cells were also noted in all animals fed Senecio powder. In swine a daily intake of Senecio powder produced some enlargement of nuclei of some hepatic cells and also enlargement of nuclei of proximal convoluted tubules of the kidneys. There was also slight epithelialization of the alveoli of lungs.

In rats trials were conducted for acute and chronic Senecio moorei poisoning. In chronic poisong Senecio powder was incorporated into basal diet making up from 1% to 4% of the ration. Tumours developed mainly in the liver. Most hepatic tumours were hepatomas (hepatocellular carcinomas), bile duct adenomas and fibromas. There was no any metastasis observed.

In sheep, rabbits and rats, trials using Adenia volkensii material produced lesions ranging from congestion to haemorrhages of various internal organs of these animals.

An exploration into the toxic principles contained in Senecio moorei and Adenia volkensii showed that Senecio moorei contained three basic compounds (alkaloids). Unfortunately these bases were not purified. Adenia volkensii was found to contain a high concentration of hydrocyanic acid, either as free or more likely in form of cyanogenetic glycoside.

The study suggests the need for additional research on tropical poisonous plants in relation to animal and human diseases and further investigation into the basic compounds present in Senecio moorei aimed at purification of the compounds.

PART ONE

I N T R O D U C T I O N

PART I INTRODUCTION

Poisonous plants cause considerable annual loss of livestock in East Africa. Despite this, plant toxicology was not regarded as of any economic importance until recently, when there was a declared policy of improvement of livestock industry in all the East African territories by introduction of exotic breeds in different areas of these countries.

Plant poisoning is due to either accidental ingestion of material eaten along with grass or to wilful consumption of poisonous plants when common pasture is dry, while most of the poisonous plants remain green all the year round.

Animals do not readily eat poisonous plants. Plant poisoning is frequently seen in transport and draft bulls, especially when they are overworked and given too little opportunity to graze. It is also more likely to occur in animals which have been moved from a different part of the country to another. Fresh importations are unfamiliar with the strange vegetation of their new surroundings. Acclimatisation in herbivorous animals induces a sense of discrimination between edible and non-edible plants.

For the purpose of this study, two species from two different genera, namely Senecio and Adenia were experimented on. An attempt was also made to identify the toxic principle where possible.

SENECIO

The genus Senecio, comprising of over 1,250 different species distributed all over the world, is the largest genus of the flowering plants belonging to the natural order compositae. Nearly 50 varieties (Verdcourt et al. 1969) are widely distributed in East Africa. Many of these species have been shown to contain alkaloids of the pyrrolizidine group. Similar alkaloids have been found in the genera Heliotropium and Trichodesma (Boraginaceae) and several species of Crotalaria (Leguminosae). These alkaloids, many of which have been investigated, are well known for their poisonous properties.

The Senecio plants and their alkaoids are of great interest in biology and medicine all over the world and particularly in South Africa (Watt et al. 1962), where they have been known for many years to produce liver damage in animals and man. Poisoning in man (Stein 1957) has occurred by contamination of the cereals and by the use of the poisonous plants in traditional medicine. In the West Indies (Rhodes 1957) they have been an important cause of cirrhosis of the liver in man.

The alkaloids are of theoretical interest because of the long lasting effect of single doses, possibly related to cell division in some tissue (McLean 1970). This effect has lead to the study of their action as antitumour substances. It may also be the basis of their disputed action as carcinogens. There is therefore, justification for the study of the basic mechanism responsible for this effect.

Ingestion of certain Senecio plants has been associated with disease conditions in horses and cattle all over the world (Sapeika 1952). These conditions have been referred to with various names such as:- "Molteno Sickness", "Dunziekte" in cattle and horses and "Bread poisoning" in man, in South Africa (Watt et al. 1962), "Winton" disease in New Zealand (Gilruth 1902), "Pictou" disease in Canada (Pethick 1906), "Walking disease" in North-western Nebraska (Van Es et al. 1929), "Veno - occlusive" disease in the West Indies (Bras et al. 1956), "Zda'r" disease of horses in Czechoslovakia (Vanek 1958), "Schweinsberger disease" of horses in Germany, "Sirasyke" disease of cattle in Norway (Hjelle 1959) and "Ragwort poisoning " in Britain.

All species of Senecio should be regarded with suspicion as being poisonous (De Waal 1941). In view of this, Senecio moorei R.E. Fries, which is so abundant in the Nyandarua District of Kenya (Fig. 1,a and b) was considered suitable for experimental studies. Apart from this the young shoots of this species have been suspected of causing death in cows immediately after long rains in the area where the plant grows in great abundance (Mugera 1971) and there were no experimental data to indicate whether the species is toxic or non - toxic to livestock.

Senecio moorei (Fig. 2) is a tall much branched herb with a woody base reaching about 1.2 - 1.8 metres high. The leaves are up to 10 cm. long by 1.4 cm. wide with a sessile base or a short, winged stock, a pointed tip and a serrated margin. When young they are covered with white wooly hairs, but these are lost as the leaves mature.

There are numerous yellow flower heads on 1.4 cm. stock arranged in a much branched inflorescence. The strap-shaped outer florets are about 13 in number and there are about the same number of bracts which bear black hairs and have papery margins.

The plant is widely distributed in Kenya between the altitudes of 2,000 and 3,000 metres above the sea level. It is very abundant around the Kinangops, Mau-Narok, Gilgil, T. Falls, Londiani, Aberdare National Park, Uplands, Molo and Kericho areas of Kenya.

ADENIA

The genus Adenia belongs to Passifloraceae family which has about 12 genera with over 500 species spread over the warmer parts of the world but largely American. In East Africa Adenia species are found in thorn bush, scrub and rocky slopes between 1,000 and 2,000 metres above sea level. The family consists of herbs and shrubs with alternate, generally stalked, lobed and stipulate leaves.

In 1903 Herbert, quoted by Watt et al. (1962), noted that in passion flower family, there is a large genus Adenia Forsk which include fifty species of herbs or climbing undershrubs occurring in Tropical Africa, Asia and Australia. Rendle (1959) recorded that Adenia contains about eighty species one of which Adenia globosa, a native of desert country of Tanganyika Territory, is an exception to the usual climbing habit, having very small leaves. Verdcourt and Trump (1969) listed the following species which occur in East Africa.

A. lobata Engl.

A. gummifera Herv.

A. schweinfurthii Eng.

A. volkensii Harms.

Several species of Adenia are known to contain hydrocyanic acid, either free or more usually in form of cyanogenetic glycoside (Watt et al. 1962). From a few species a toxalbumin, modeccin has also been isolated. Several species of this genus have been used by Africans for treatment of various disorders (Watt et al. 1962). Adenia volkensii is reputed by the Akamba tribe of Kenya to be excessively poisonous and has been implicated in cases of homicides (Verdcourt et al. 1969). There has been a lot of superstition regarding this plant.

Adenia volkensii (Fig. 3) is a subsucculent herb of about 30 to 150 cm. tall arising from a tuberous rootstock which grows rather deeply in the soil. The leaves are long and broad, deeply 3 - 5 lobed. The lobes may again be divided. Rarely the leaf is undivided. The flowers are small, bell-shapped, green or yellow in colour with brown or purplish spots. Fruits are large, rather like tomatoes. They are green when unripe and red on ripening.

The plant is common in the thorn bush in Embu, Kitui and Machakos Districts of the Eastern Province of Kenya (Fig. 1,a) and elsewhere in scrub-rocky slopes of East Africa between the altitudes of 1,000 to 2,000

metres above sea level. The toxicity of this plant in animals and man has been known by the Akamba people for a long time.

The tuberous part of the plant is used as an abortifacient by the Mbere and the Akamba tribes of Kenya (Verdcourt et al. 1969). In Isiolo and Maralal areas of Kenya the indigenous people use powder made from the tuber to poison hyenas. The powder is incorporated in meat baits.

Adenia volkensii was suggested for experimental purposes because of its unique importance among the Akamba as a highly toxic plant. The common occurrence of this plant particularly in the Eastern Province of Kenya also necessitated the research. It was recognized well in advance that no work had been carried out to establish the poisonous principle.

The two species of plants described above were collected from different parts of Kenya (Fig. 1,a) and samples of these plants with leaves, flowers and fruits forwarded to the East African Harberium, Nairobi, for identification.

The impetus for research on these species has been the recognition that:-

(a) No available record of their toxicity has been found, (b) all species of Senecio should be regarded as poisonous unless proved otherwise, (c) no work has so far been done to investigate

the poisonous principle of *Adenia volkensii* Harms, (d) other species belonging to the genera of the above species have been proved to be highly toxic to both animals and man and (e) strong evidence points to *Senecio* as being carcinogenic.

The purpose of this research is three-fold:-

(i) To study *Senecio moorei* toxicosis in Rats, Pigs and Cattle,

(ii) To study *Adenia volkensii* toxicosis in Rats, Rabbits and Sheep and (iii) to carry out preliminary investigation into toxic principle of:-

(a) *Senecio moorei*, R. E. Fries

(b) *Adenia volkensii*, Harms.

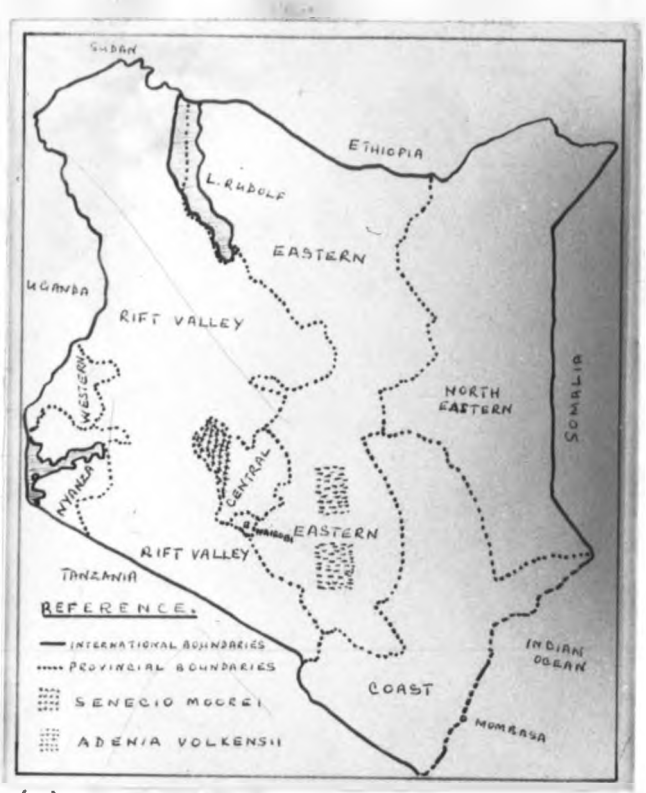

Fig. 1 (a) Map of Kenya showing provincial boundaries and areas where Senecio moorei and Adenia volkensii were collected.

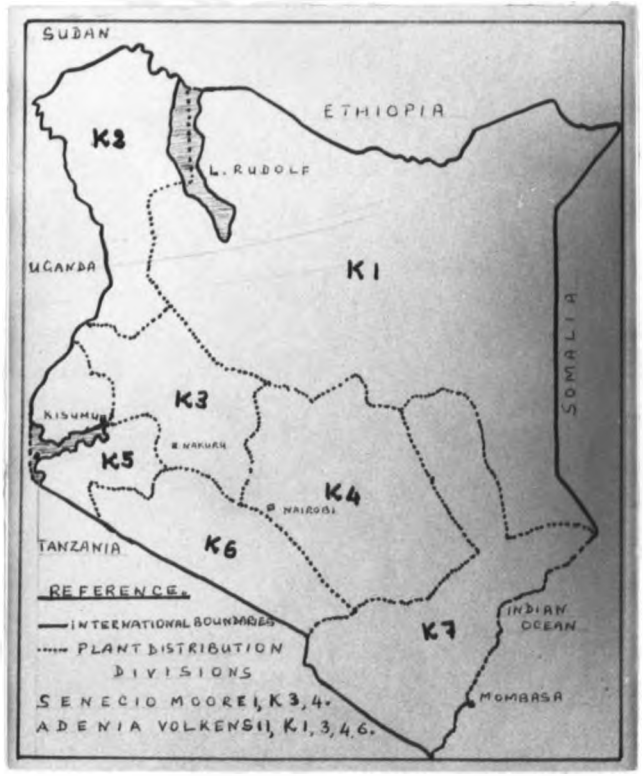

Fig. 1. (b) Map of Kenya showing geographical distribution of Senecio moorei and Adenia volkensii.

Fig. 2. Senecio moorei R.E. Fries (Mau-Narok ragwort).

Fig. 3. Adenia volkensii Harms (Kiliambiti).

PART TWO

REVIEW OF THE LITERATURE

PART II REVIEW OF THE LITERATURE

SECTION 1. SENECIO

Senecio as Medicine

Various species of the genus Senecio have been used in herbal medicine by different African tribes, particularly in the Southern and Eastern Africa.

Smith (1888) wrote that the Mfengu and Xhosa tribes applied as poultice of the leaf of *Senecio concolor D.C.* to cuts, wounds and swellings. Hot decoction was sometimes used for the same purpose. The latter tribe applied a paste of the leaf to cuts and wounds to keep down the inflammation and swelling. They also applied paste of the leaf of *Senecio deltoideus Less.* to sore eyes.

Watt (1889-96) recorded the use of seeds of *Senecio quinquelobus D.C.* as an indigenous colic remedy in the Kanawa. The Zulu used an infusion of the same species as an enema and orally in influenza. Merck, quoted by Watt et al. (1962), referred to the use of *Senecio vulgaris L.* for dysmenorrhoea and amenorrhoea. In United States the plant has been used as a diaphoretic, a diuretic, a tonic and an emmenagogue.

The Zulu used a decoction of the root of Senecio fraudulentus Phillips and Smith, as a remedy for palpitations and for phthisis while the Kwena and Tswana used the plant as remedy for coughs and difficult breathing (Watt et al. 1962). Dragendorff (1898) stated that the herb of Senecio haworthii Sch. Bip. had been used for chest complaints.

According to Bryant, quoted by Watt et al. (1962), the Zulu took a decoction of the leaf and stalk of Senecio speciosus Willd. for pleurisy and other pains in the chest. Phillips, also quoted by Watt et al. (1962), mentions the following senecio species which the Southern Sotho used either as colic remedy; emetic or for colds and other respiratory troubles. Senecio albanensis D.C. roots were used as colic remedy. Senecio brachypodus D.C. - infusion was used as medicine for colds and other respiratory troubles. A decoction of the root of Senecio dregeanus D.C. was used as an emetic and remedy for madness. Senecio gerrardi Harv. served as emetic in biliousness. Senecio macrocephalus D.C. var. hirsutissimus Harv. was used to relief colic. A decoction of the root of Senecio rhyncholaenus D.C. was drunk to relief colic, Senecio serratus Sond. was used to wash persons suffering from swollen limbs and internal tumours. The same tribe used medicinally Senecio subcoriaceus Schltr. and Senecio tanacetoides Sond. In 1926, Senecio aureus was actually official in National Formulary (U.S.A.), (Sapeika 1952).

Steyn (1933) stated that the powdered root of Senecio venosus Harv. was used by the Manyika as a snuff for the relief of headache. Bally (1937) mentioned the use of Senecio stuhlmannii Klatt by the Shambala as a remedy for ulceration. In Notes on Native Medicinal Plants in East Africa (1938) he also mentioned the use of Senecio mranguensis O. Hoffm. by the Haya as remedy for yaws, for syphilis and for gonorrhoea. De Wildemann, quoted by Watt et al. (1962), listed some 14 senecio species which had been found to produce a cicatrizing effect in skin diseases. He also stated that in Belgian Congo (Kivu) Senecio ruwenzoriensis S. Moore had been used to reduce inflammation in the limbs.

Cook et al. (1950) wrote that Sutos administer Senecio asperulus D.C. to charm away nightmares in children; Quenas and Chunas administer a milk concoction of the root of another Senecio plant to infants as a stimulant; Sutos smoke the leaf of Senecio phyncholaenus D.C. or inhale the smoke from burning it to treat cold; they drink a concoction of the root for colic, and mix the leaf of Senecio erubescenus D.C. with their tobacco. Zulu use different Senecio plant infusions as blood purifiers in syphilis, chest pains, swollen gums etc.

Potter and Wren, quoted by Cook et al. (1950), noted that senecio plants were sold in Great Britain for treatment of colds, coughs, sciatica, pains of limbs and other disorders. According to Grossweiler

(1953) Senecio sereti De Willd. is used medicinally by the African in Angola. Nazaye, quoted by Watt et al. (1962), reported the used of Senecio abyssinicus Sch. Bip. leaf in Belgian Congo to stimulate the healing of wounds and sores and for eye diseases.

Senecio as Poison to Humans

Ingestion of certain senecio plants has long been known to cause human poisoning. Cases of "bread poisoning" in human beings have been described in South Africa (Steyn 1933). Senecio species growing wild on corn fields eg. Senecio ilicifolius Thumb. in the S.W. Cape district have been harvested with the corn and through improper winnowing have led to poisoning.

Albertijn, quoted by Willmont et al. (1920),drew the attention of the Union Government, Health Department, to certain cases of sickness of obscure causation occuring in George district. Investigations showed that Senecio ilicifolius and Senecio burchellii grew abundantly as weeds in the district and the patients might have consumed wheat meal containing seeds of these plants. Willmont and Robertson (1920) studied a few cases of human poisoning by bread containing seeds and leaves of Senecio ilicifolius. Muir (1928) noted that the most common species Senecio ilicifolius and Senecio burchellii probably cause cirrhosis of the liver. He described the method of separating Senecio seeds from

grain before milling. Selzer and Parker (1951) reported observations of twelve cases in European and Coloured adults and six children, six of whom died. Necropsies of the six dead patients presented strong evidence that condition observed by them was caused by the consumption of bread made of inadequately winnowed wheat and contaminated with Senecio (ragwort), a common plant. Stein (1957) described four cases similar to senecio poisoning and argued that although there had not been any evidence to suggest that his patients had taken any herb or plant substance such as senecio, it was common for African children to be given witch-doctors medicine and that he could not exclude the possibility that toxic substances especially witch-doctors herb were important aetiologically.

Outbreaks of acute intoxication and liver cirrhosis due to pyrrolizidine (Senecio) alkaloids have been endemic in the West Indies (Bras et al. 1956). They have also occurred sporadically in widely scattered parts of the World (Hill 1960). Bras and Hill (1956) suggested for the first time the use of "Hepatic Veno-occlusive Disease" (V.O.D.) for the above conditions originally reported in Jamaica as "Serous hepatosis and collagenosis". These intoxications and liver lesions are characterized by acute liver enlargement and ascites, often progressing to early cirrhosis. One of the main pathological features of this liver condition is partial or complete occlusion of the centrilobular

veins and the smaller hepatic tributaries. Chiari (1899) showed that the syndrome is the result of a primary thickening, occlusive thickening, of the intima of the central and sublobular hepatic veins and that it occurs independently of changes in the surrounding tissue.

As early as 1939 this condition was described in Germany (Wurm 1939) and in Egypt (Hashem 1939) all quoted by Hill (1960). McFarlane and Branday (1945), in Jamaica, described ascites with hepatomegaly in 18 patients. They postulated a toxic aetiology but observed that dietary factors may also play a role in the causation of the condition. Hill et al. (1951) found ascites with hepatomegaly and oedema of the legs, a prevalent clinical syndrome in Jamaica, with constant histopathological findings in the liver. Bras et al. (1954) described veno-occlusive disease of the liver with non-portal type of cirrhosis occurring in Jamaica. Bras and Hill (1956) stated that the resemblance between veno-occlusive disease of the liver and senecio poisoning in cattle was striking. They could hardly distinguish slides from a cow in England thus afflicted from those of veno-occlusive disease in man. They suggested that "Bush teas" widely taken by the Jamaica population for their alleged medicinal value may play an important toxicological role.

Senecio as Stock Feed

Vardiman (1952) in Texas reported on an experiment with silage made from the green plants of Senecio riddellii which were proved to be

toxic. The silo was filled with the green chopped plant and made thoroughly wet with molasses and water. After being closed for 114 days it was opened and fed to calves for a period of three months. It proved to be non-toxic. The results demonstrate that under suitable conditions microbiological fermentation can destroy the toxicity of the pyrrolizidine alkaloids.

Senecio as Poison to Animals

Seneciosis in animals has been known for many years and has a world wide distribution. As early as 1902, Gilruth (1902) was led to examine the effects of Senecio jacobaea of New Zealand. It had been observed that cattle on the station affected by "Winton" disease had been eating this plant. His feeding experiments on healthy calves with a ration containing this plant confirmed that Senecio jacobaea poisoning produced symptoms typical of "Winton" disease.

Cattle were among the earliest animals to be investigated when farmers in Nova Scotia noted that their cows fell ill after eating the imported weed Stinking Willie (Senecio jacobaea). A number of investigations carried out in 1882 under government auspices failed to connect the disease with the weed and cirrhosis of the liver was therefore put on the list of contageous diseases. Animals affected with it were slaughtered and the building in which they were kept disinfected. Pethick (1906) carried out an investigation of the subject

and showed beyond doubt that poisoning with Senecio jacobaea was the cause of the disease known as "Pictou" disease in Canada. It was also noted that sheep could feed on ragwort with comparative impunity, though they also suffered after sometime and the flesh finally assumed a yellowish tint (jaundice?). Rutherford, quoted by Cushny (1911), confirmed that sheep ate ragwort with impunity whether the plant was in the dry or green state. Cattle refused to eat it in the green state and the poisoning seen in Canada arose only from the dried ragwort in the hay.

In South Africa various species of senecio have from time to time been regarded as causing disease conditions to animals. Such conditions as "Molteno" cattle sickness, 'Straining' disease in cattle, "Dunziekte", "Stomach Staggers", "Grass Staggers", "Ragwort" poisoning and "Senecio Cirrhosis" have been ascribed to the eating of senecio.

Robertson, quoted by Watt et al. (1962), showed that senecio, called at that time Senecio latifolius D.C. was toxic to oxen and horses and attributed the natural disease to this cause and also to Senecio burchellii D.C. Dixon (1906), produced condition indistinguishable from Molteno disease in horses by feeding Senecio latifolius. He stated that this disease rendered horse breeding impossible in the East London District of South Africa. Verney, quoted by Watt et al. (1962), fed a horse for fifty-six days on ragwort but he was not able to produce the

clinical symptoms and on post-mortem of the horse could not find conditions typical of dunziekte. In his discussion he did not consider the negative results as conclusive against the view that the plant was the causal agent of the dunziekte.

Theiler (1918) found it difficulty in accepting the senecio causation of dunziekte among other things but stated that dunziekte must be looked upon to be a disease which is caused by the consumption of a plant poison of specific nature and in this respect resembles other specific diseases that are connected with pasture and that it should be classified with diseases of vegetable origin such as Geeldkkop in sheep, Styfziekte in cattle and Jagziekte in horses which are well defined morbid entities. In 1929, the same author maintained that although he was able to produce a condition closely resembling dunziekte by feeding Senecio latifolius D.C. (so called at that time), there was essential difference in the liver pathology between senecio poisoning and dunziekte; horses refuse to eat the plant when it is offered to them and that large quantities of senecio are necessary over long period of time to produce experimental senecio poisoning. Steyn (1929) reported on feeding experiments of 75 species. Senecio latifolius D.C. fatally poisoned sheep with as little as 5 Gm. of dry plant per day for 16 days. A horse died after receiving 900 Gm. of dry material in eight days and a yearling steer was killed by 340 Gm.

given in 6 days. In 1931 he gave results of experimental feeding of 49 plant species to various animals. He proved that Senecio isatideus was poisonous to domestic animals. De Kock et al. (1931) recorded that dunziekte was so severe and so prevelent in the Kokstand district that horse breeding had been abandoned on many farms. On basis of the results of their experimental investigations they made the following statement:-

> "It may be stated that symptomatically and pathologically there is a very close resemblance between senecio poisoning and dunziekte, infact cases were produced by drenching senecio which could not be distinguished from dunziekte".

Steyn (1933) recorded typical senecio poisoning in a horse on farm in the Humansdorp district under circumstances which made it clear that Senecio burchellii was the causative agent. In 1941 he further noted that Senecio sceleratus Schweickerdt was toxic, and described 16 other species of senecio in South Africa which were known to be toxic to all kinds of animals. Ingestion of large quantities within a short time resulted in acute poisoning, the animals dying in few hours or days. Chronic poisoning occurred when animals repeatedly consumed small amounts. De Waal (1941) noted that the genus senecio is widely distributed in the Union and that some 300 species which occur there sometimes cause heavy stock losses. Horses are the main victims but cattle and sheep are also destroyed. He added that as no satisfactory

remedies were available large scale eradication measures were recommended. Van der Walt (1944) confirmed that Senecio isatideus was fatal to sheep which took 2.25 kg. of fresh flowering plant.

Senecios were incriminated as a cause of cattle deaths in the Eastern Districts of Rhodesia as long ago as 1908. Thus Sinclair, quoted by Shone et al. (1965), reported that a large number of cattle deaths occurred on the farm Mountain Home, Umtali when animals grazed upon old lands on which Senecio sceleratus was very plentiful. Hesketh, also quoted by Shone et al. (1965), reported that the mortality in 1944 had reached alarming proportions and that in 1941 the mortality had also been heavy. He suspected Senecio sceleratus or Bracken or both to be responsible. Shone and Drummond (1965) noted that in recent years annual mortality had been considerable and on one farm alone 50 head of cattle in one year died as a result of eating this plant. They also stated that in the Eastern Districts, chronic form of the disease known in South Africa as Molteno Cattle Disease was rarely encountered.

Apart from Canada and South Africa Senecio poisoning had been reported from many other countries. As far back as 1787 some farmers in Great Britain are said to have believed Senecio jacobaea to be harmful to livestock. Stockman (1917) described an outbreak of

the disease in a heard of cattle which was due to the consumption of hay contaminated with Senecio jacobaea. Thompson, quoted by Bull et al. (1968), reported the death of one of seven young cattle 2 months after they had been placed on a second hay in which a vigorous growth of the plant had occurred but had been mowed and left on the ground. Knowles (1926) reported an outbreak in Lincolnshire in which 15 cattle and 8 horses died. The pastures were infested and the hay being fed to the animals was contaminated with the plant. Craig et al. (1930) reported occurrence of the disease in cattle and in horses in Ireland. Other outbreaks had been reported from time to time mainly associated with consumption of contaminated hay. Reynolds (1936) reported the occurrence of ragwort poisoning in cattle in Pembrokeshire. He found that cattle and horses do not eat the weed except possibly during a dry spring when the green plant may show in the pasture before the grasses. The real danger was from contaminated hay. Bisset (1936) also reported an outbreak of the disease in cattle. Evans and Evans (1949) reported on the poisoning of farm animals in Wales by the consumption of Senecio aquaticus. Betty and Markson (1954) described an outbreak in heard of cattle and the use of liver biopsy as an aid to diagnosis. In this outbreak the hill pastures were heavily infested with Senecio jacobaea and the outbreak was confined to the heifers and dry cows which grazed there. Forsyth (1954) says that ragwort alone probably causes more annual loss of livestock in Great Britain than all

other plants put together. Cockburn et al. (1955) reported on an outbreak of acute poisoning in cattle by the consumption of Senecio jacobaea which was a contaminant of feed cubes prepared from lucerne. The first death occurred within a fortnight of commencement of feeding the cubes to the stock. Donald and Shanks (1956) described an outbreak of the disease in cattle which had been fed on silage containing ragwort. The first case occurred ten weeks after the feeding had been started.

In the United States of America, "Walking disease" of horses had been a problem of great economic importance after about 1912 in north-western Nebraska and in parts of Wyoming and Colorado adjacent to Nebraska. The disease was enzootic in distribution and seasonal in occurrence. It was characterized by a cirrhosis of the liver as well as by its clinical signs. Van Es et al. (1929) made a detailed study of the disease and carried out experimental feeding of rabbits and horses with the two species of senecio, Senecio integerrimus and Senecio riddellii, commonly growing in the area involved. They produced the clinical signs and the pathological changes in the liver in horses fed on Senecio riddellii. Their experiments with Senecio integerrimus gave indefinate results. Senecio poisoning has also been of economic importance in the Big Bend area of Texas. Mathews (1933) described this disease which in 1927 had had been at its worst and had involved several thousand cattle with an average mortality between 10 and 12 per cent.

In Germany, "Schweinsberger disease" of horses was known to be a cirrhosis of the liver which was enzootic in certain areas, especially in swampy country. Description of the disease go back as far as 1886. Its aetiology remained undetermined for many years although some associated it with plant poisoning. Opperman and Ziegler, quoted by Bull et al. (1968), regarded this disease as a chronic form of infectious anaemia. It was recognized by many however, to be indistinguishable from senecio poisoning of horses described by Gilruth and later by others. Koehler, quoted by Bull et al. (1968), showed that this disease was due to the consumption of Senecio vernalis mainly as a contaminant of hay. Hupka, also quoted by Bull et al. (1968), confirmed the aetiological significance of Senecio vernalis which was a contaminant of lucerne hay. This disease which had been shown to belong to the list of senecio poisoning occurs in Bavaria, Germany. "Zd'ar disease" of horses in South Bohemia, Czechoslovakia was found by Vanek (1958) to be due to chronic poisoning from the consumption of Senecio erraticus Spp. barbaraeifolius. As early as 1933 seneciosis was reported in Norway. Dybing and Hjelle, quoted by Bull et al. (1968), reported liver disease in calves as due to the consumption of Senecio aquaticus. Hjelle (1959) noted that seneciosis constitutes the most important plant poisoning in cattle in Norway.

In Australia there has been few reports of senecio poisoning in livestock. Murname, quoted by Bull et al. (1968), recorded the occurrence

of losses of dairy cattle on one property in the South Gippsland district of Victoria where Senecio jacobaea was plentiful. Hurst (1942) mentions that several species of senecio, including Senecio jacobaea have been suspected of causing losses to individual animals but no serious outbreaks of disease have been reported.

Thorold (1953) reported several outbreaks of senecio poisoning at Timau, Kenya. The animals had been kept in paddocks where Senecio ruwenzoriensis was plentiful. Symptoms described and the liver sections showed the typical changes associated with senecio poisoning. He also suspected Senecio mooreii to be poisonous to livestock.

Animal Research

Various workers such as Gilruth (1902) in New Zealand, Theiler (1918) in South Africa, Pethick (1903-06) in Canada and Van Es (1929) in America proved that certain species of senecio were responsible for disease conditions but there was always a certain amount of doubt as to the exact nature of the poison.

Cushny (1911) carried out experiments with two alkaloids isolated from material named Senecio latifolius D.C. from the cape namely, senecifoline and senecifolidine and showed that the symptoms, post-mortem findings and histological changes in the liver animals poisoned by these two alkaloids very closely resembled those found in "Molteno" disease. He expressed the opinion that there was no doubt that Molteno

disease is a chronic senecio poisoning. The same author worked on the pharmacology and toxicity of other senecio alkaloids. By injection of senecifoline nitrate into rats he produced mortality and lesions resembling those in natural cases. Burkey et al. (1933) extracted crude crystalline mass from Senecio reddellii and fed it to a horse. They developed the characteristic sysmptoms of "Walking" disease. Davidson (1935) injected retrorsine subcutaneously into rats with the object of studying the pathogenesis of the lesions. Doses of 0.007 to 0.25 Gm. were used in order to vary the severity of the lesions.

Chen et al. (1940) showed that the intravenous injection of lethal or near lethal doses of seneciphylline, an alkaloid from Senecio spartioides, in mice, rats and guinea pigs is followed by tonic convulsions. The post-mortem lesions revealed periportal necrosis, leukocytic infiltrations and vacuolation of the parenchymatous cells. There was also cloudy swelling with venous and glomerular congestion. Platphylline appeared to have an atropine-like action. Harris et al. (1942) described the action of senecionine, intergerimine, jacobine, longilobine and spartioidine especially in the liver. They determined the median lethal doses of these alkaloids in mice by intravenous injection. Deaths occurred in 24 to 96 hours. They also determined the acute toxicity of monocrotaline and retronecine in mice. Intravenous injection of a large dose of retronecine was followed by either death or complete

recovery. These same authors studied three additional Senecio alkaloids, namely, isatidine, pterophine and sceleratine. Their acute toxicity was determined in mice by intravenous injection. Pterophine proved to be the most and isatidine the least toxic. Harris et al. (1943) reported that carthamoidine, an alkaloid from Senecio carthamoides, in suitable doses produced slow death and necrosis of the liver of mice when injected intravenously. Rose et al. (1945) studied the effect of hepatotoxic alkaloids on the prothrobin time of rats and noted that senecionine, retrorsine, pterophine, spartioidine and monocrotaline in sufficient doses prolonged the plasma prothrobin time of rats. Wakim et al. (1946) reported that intravenous administration of senecionine to four rhesus monkeys led to development of extensive necrosis of the liver of three of the animals and fatty degeneration of all.

Rosenfeld et al. (1949) wrote that the administration of crude alkaloids of Senecio riddellii to mice produced acute intoxication followed by remission, recurrence of the symptoms and death. The toxic dose was determined by intraperitoneal and oral administration of the alkaloid. The feeding of the plant produced typical symptoms of chronic poisoning similar to that observed in cattle. Sapeika (1950) reporting on certain pharmacological actions of pterophine, a senecio alkaloid, obtained from Senecio pterophorus and Senecio ilicifolius noted that in frogs the injection into the ventral lymph sac of pterophine, 140 mg. per Kg. body weight, did not cause death. In anaesthetized cats,

intravenous injection of 20 mg. per Kg. produced a fall of blood pressure, rapid shallow respiration, stimulation of the uterus and in rabbits, elevation of blood sugar. The blood pressure of rats and dogs was also lowered by intravenous injection of the drug.

Cook et al. (1950) observed liver tumours in three albino rats which survived for more than 8 months of intermittent feeding with alkaloids of Senecio jacobaea. They discussed the possible bearing of these results on the aetiology of primary liver tumours frequent among the Negro population in South Africa, in the light of the indiscriminate use of senecio plants for treatment of numerous disorders. Selzer et al. (1951) dosed orally rats on normal or low protein diets with retrorsine hydrochloride and noted that the toxic effect involving both the liver parenchyma and the central hepatic veins produced picture of centrilobular haemorrhage, necrosis, proliferation of the endothelium of the central and hepatic veins. Protein deficiency enhanced the toxic effects of retrorsine. Moraes (1951) showed that Senecio brasiliensis was potentially dangerous. The chemical and biological properties of brasilinecine, an alkaloid from this plant, were detected and the median lethal dose for rats and mice ascertained. Smit (1952) determined the toxicity of retrorsine, isatidine and pterophine for white rats after subcuteneous injection. He found that sex influenced the susceptibility of rats to retrorsine and isatidine profoundly. Sapeika et al. (1953) described the hepatic lesions produced in rats by acute poisoning

with senecio alkaloid pterophine. They noted that the ascorbic acid content of the liver becomes increasingly diminished with progressive damage of the tissue.

Schoental et al. (1954) reported that 58 rats survived longer than 10 months of treatment with senecio alkaloid from Senecio jacobaea Linn. retrorsine and isatidine. Forty five of these rats showed changes in the liver ranging from hyperplasia to neoplasia. Metastasis were found in one rat that was treated with isatidine. Schoental (1955) described blood changes in rats treated with senecio alkaloids. She suggested that young plants containing pyrrolizidine alkaloids in form of N-oxides are more palatable and readily consumed by livestock, which might produce pathological changes later. Campbell (1956) showed that the alkaloid seneciphylline, isolated from Senecio jacobaea Linn. was potent specific hepatotoxic agent in poultry. He also discussed the demonstration of the carcinogenic properties of seneciphylline in certain Bantu tribes who subsist on deficient diets and who may drink senecio infusion as a component of native medicine. In his studies on the influence of sex hormones on avian liver and the protective effect of oestrogen determined by bromsulphthalein liver function test, he noted that previous treatment with diethylstilboestrol protects the male birds liver to a considerable degree against the hepatotoxic effect of the alkaloid seneciphylline.

McKenzie (1958) describing some pharmacological properties of pyrrolizidine alkaloids noted that platyphylline, jacobine, senecionine, seneciphylline and jaconine are less potent inhibitors of tonus than epinephrine and less active anticholinergic than atropine on rats' isolated ileum. Sedlmeier et al. (1959) observed that the first visible changes produced by prolonged feeding of Senecio vulgaris and p-dimethylaminoazobenzol were vacuolation of the parenchymatous cells, nuclear inclusion, proliferation of bile-ducts, regenerative hyperplasia, cirrhotic changes and deposition of pigment in the internal organs. Schoental et al. (1959) described their further observations on the subacute and chronic liver changes in rats after a single dose of various pyrrolizidine (senecio) alkaloids. They discussed the origin and fate of the enormous parenchymal cells characteristic of the subacute lesions.

Dybing et al. (1959) reported the feeding of male rats with 1% of pulverized dried Senecio aquaticus. They noted that after administering for brief intervals the concentration had no effect on the animals but when given for longer intervals (7-9 months) nine tenth of the animals died with severe liver lesions. Thorpe and Ford (1968) studied the sequential changes in the livers of 5 calves fed several levels of ragwort (Senecio jacobaea) in their diet by series of biopsy. Parenchymal megalocytosis, hepatic fibrosis and veno-occlusive lesions developed in all calves. The histochemical enzyme studied by Ford and

Ritchie (1968) showed that there was little if any relationship between the progress of the liver changes and the pattern of changes of the serum enzyme concentration.

Chemical Research

Chemical studies on Senecio plants were focused on isolation and identification of the chemical constituents of the alkaloids. Senecio alkaloids contain one nitrogen atom only; although the number of carbon atoms varies the predominating figure being eighteen. The content of hydrogen and oxygen atoms varies considerably. Some alkaloids have been isolated from more than one plant.

Grandval and Lajoux quoted by De Waal (1939), isolated the first alkaloid of this genus from Senecio vulgaris, the common groundsel, and named it senecionine. Manske (1931) isolated an alkaloid retrorsine from Senecio retrorsus which marked the beginning of a new era in the investigation of various alkaloids. He assigned to this alkaloid the formula $C_{18}H_{25}O_6N$. Barger and Blackie (1936) confirmed his work. Retrorsine, however, was found to be present in Senecio latifolius D.C. (Barger et al. 1935) and in Senecio glaberrhimus. (Blackie 1937). Manske (1931) made also the first important invstigations of the alkaloids of Senecio jacobaea and obtained a compound, m.p. 22-224°C., which analysed poorly for $C_{18}H_{23}O_5N$. In 1939, he wrote that senecionine is the main alkaloid of Senecio integerrimus but a small amount of a new alkaloid, integerrimine, was also found. Senecio longilobus contains longilobine and

Senecio riddellii contains riddelline, both alkaloids being new. In addition to the main alkaloid of Senecio spartioides, which was identified as seneciphylline, a minor base apparently new was also obtained.

Watt (1909) isolated two alkaloids, senecifoline and senecifolidine from Senecio latifolius. In 1909 the identification of species of senecio was very unsatisfactory and it is probable that at that time the name Senecio latifolius D.C. included Senecio latifolius, Senecio retrorsus, and Senecio barbellatus. Hence it is impossible to say from which species of senecio Watt isolated the above alkaloid (Steyn 1934). Orechoff (1935) isolated from Senecio platyphyllus the alkaloid platyphylline which can be hydrolyzed into platynecine and platynecic acid. Blackie (1937) reported the isolation of a new alkaloid from Senecio isatideus D.C. which he called isatidine. He found isatidine in very appreciable quantities (1.14 per cent) in Senecio isatideus and also isolated retrorsine from this plant. This retrorsine proved to be identical with that isolated from Senecio retrorsus but was present in the former in smaller quantities (0.15 per cent). Hosking and Brandt, quoted by Steyn et al. (1941), isolated the alkaloid jacobine from Senecio jacobaea growing in New Zealand. They stated that this alkaloid " is very probably identical with the alkaloid jacobine previously isolated from ragwort by Manske."

De Waal (1939) confirmed the findings by Blackie by experiments dealing with isolation of isatidine from Senecio retrorsus and Senecio isatideus, and in 1940 described the isolation of the alkaloid platyphylline from Senecio adnatus D.C. which he reported was in conformity with that isolated by Orechoff (1935) from Senecio platyphyllus D.C. The same year in his chemical investigation upon the senecio species responsible for bread poisoning, he isolated the alkaloid senecionine from Senecio ilicifolius Thumb, and another new alkaloid rosmarine from Senecio rosmarinifolius Linn. He also showed that platyphylline was the active principle of Senecio adnatus D.C. In 1941, he reported on the chemical investigation and isolation of three alkaloids from Senecio sceleratus sp. nov. Schweikerdt. The three alkaloids isolated were isatidine, retrorsine and a new alkaloid for which the name sceleratine was proposed. In "Notes on Isatidine, Rosmarinine and Pterophine and on the structure of their Necines and Necic Acid" the same author wrote that of all the 18 senecio species known, on hydrolysis they produce a "necine" base and a "necic" acid. De Waal et al. (1941) recorded that toxicological studies proved that Senecio sceleratus was extremely toxic to sheep and that the ground plant contained 0.17% retrorsine, 0.11% sceleratine, a new alkaloid, and 0.05% isatidine.

Novelli et al. (1945) isolated an alkaloid from the leaves of Senecio brasiliensis, on alkaline hydrolysis it gave retronecine and an acid. Briggs et al. (1948) isolated a new alkaloid senekirkine from the bark and leaves of Senecio kirkii. Adams et al. (1949) isolated two

alkaloids alpha and beta-longilobine from Senecio longilobus and found that their empirical formulas were $C_{18}H_{23}O_5N$ and $C_{18}H_{25}O_6N$ respectively. By degration they were shown to be cyclic diesters from one mole of retronecine and one mole of dibasic acid. Sapiro (1949) showed that Senecio bupleuroides contain the same two alkaloids as Senecio retrorsus and senecio isatideus namely retrorsine and isatidine. The quantity of these alkaloids extractable from dried plant collected in the post feeding stage was retrorsine 0.16% and isatidine 0.7%. He also showed that the plant is of low toxicity compared with Senecio retrorsus and Senecio isatideus and that negative results were obtained in a physiological test. In 1953, he isolated two alkaloids namely ruzorine and ruwenine from Senecio ruwenzoriensis common in Kenya. Pretorius (1949) obtained retrorsine, isatidine and a new alkaloid paucicaline from Senecio paucicalyculatus. Early flowering plant contained over 2.5% crude isatidine and 1% crude retrorsine in the leaves and roots. Koekemoer (1951) described the preparation and properties of the N-oxides of ptatyphilline, platynecine and rosmarinine. He indicated the occurrence of the senecio alkaloids as N-oxides or their tantomeric forms and described an improved method of isolation to give yields several times greater than those previously reported. Bradbury et al. (1954) isolated two new alkaloids, jacozine and jacoline from Senecio jacobaea. The alkaloid jaconine appears to be the first recorded example of a chlorine containing alkaloid. Henning, quoted by Bull et al. (1968), described the method of isolating the alkaloids of Senecio discolour from the stem and leaves and added that paper chromatography indicated the presence of 2 major alkaloids components. The two alkaloids were identified as sonecionine and retrorsine.

Senecio Poisoning

Davidson (1953) described the clinical symptoms and the post-mortem lesions in senecio poisoning as follows:-

(a) Symptoms

"The symptoms in the affected animals are similar in all countries. There is a loss in appetite, weight and ascites may be present and the animal has difficult in standing and walking. Cerebral symptoms supervene with difficulty in maintenance of balance and a staggering gait. Marked frenzy may be noticed. Coma follows and death occurs a few days after the onset of the first symptoms. The disease is usually afebrile".

(b) Post-Mortem Findings

"Necropsy examination shows a certain amount of jaundice of the mucosae. The surface of the liver is uneven and the tissue is tough when cut by knife.

The duodenal mucosa may be hyperaemic and swollen while the rest of the bowel is usually normal. There may be petechial haemorrhages in other organs but there presence may be irregular. The liver on the whole appears to be the principal seat of the disturbance. Microscopically, various stages of the process in the liver may be seen. In the more acute and early types there is extravasation of the blood around the central veins of the lobules. These have been called "blood pools" or "blood lagoons". The liver cells in the vicinity of these areas

degenerate and disappear. In the later stages there is a definate fibrosis which Theiler has called an "interstitial hepatitis". There is a loss of the normal architecture of the liver and the cells are replaced by fibrous tissue in the midst of which there is proliferation of bile ducts. The picture is that of a cirrhosis"

SECION II. - ADENIA

Adenia as Medicine

Various species of Adenia have been used in herbal medicine by many tribes. Bryant, quoted by Watt et al. (1962), reported the use of the powdered leaf and stem of Adenia gummifera by the Zulu as an emetic in the treatment of biliansuess. He also stated that the Zulu and Renga tribes used a decoction of the root for the treatment of malaria and leprosy. The both tribes used a steam bath on boiling the leaf in water in treatment of malaria. The Zulu used the root as a tonic in convalescence.

Burtt (1926) recorded that the Africans in Transvaal used the gummy substance from Adenia gummifera as an emetic and as cosmetic pigment applied on the face. He also mentioned of the use of an Adenia species in treatment of tuberculosis. Steyn (1929) reported that the Nyanja tribe used boiled root of Adenia digitata or root decoction in local treatment of leprosy, ulcers and other skin infections.

In East Africa, the Tonga tribesmen used Adenia kirkii for the treatment of bronchities (Almeida, quoted by Watt et al, 1962). Irvine (1930) described the use of the leaves of Adenia cissampeloides for rubbing the breasts of women after child birth to promote the flow of milk.

Bally. (1937) reported the use of the tuberous stem of Adenia globosa by the Masai in the treatment of cattle for certain diseases. He also mentioned the use of Adenia gummifera by the Sukum as an antidote to arrow poisoning. The Lenge tribe uses one of the Adenia species in the treatment of infantile convulsions. Irvine, quoted by Watt et al. (1962) reported of the many medicinal uses of Adenia lobata. In "French Equatorial Africa" the roots were used with red pepper and Guinea grains for effective treatment of cancer of the nose. The leaves were used for piles and thoir juice as an enema and liniment. The leaf decoction was drunk for cough, bronchitis and fever. In the Ivory Coast an enema from the leaf extract was used as an efficient purgative during fever. An enema from the stem palp was used as an aphrodisiac, diuretic and in the treatment of gonorrhoea. In Ivory Coast the plant was also used for feverish pains, rheumatism, intercostal pains and stomach troubles.

Adenia as Food

The fruits of Adenia species resemble those of many other members of passion-flower family which are edible. Although not all,

some species of Adenia have edible fruits. Thus cases of poisoning have occurred after eating toxic fruits of non-edible species by mistake. Burtt (1926) reported that despite the toxic nature of the fruit and tuber of Adenia digitata, the Africans sometimes sucked a morsel of the plant as a thirst quencher. Few, quoted by Watt et al. (1962), said that he observed children eating the fruit of Adenia glanca which they said was very nice. Verdoorn (1938) reported that the fruit of Adenia hastata which resembles the grandanilla is edible. Verdcourt and Trump (1969) recorded that an undescribed species referred to as "diati" (Keritschoner 1728) has edible fruits. Irvine, quoted by Watt et al. (1962), wrote that Adenia has edible fruits. He added that confined women take a beverage prepared from the roots of Adenia. Liebenberg (1939) reported that Adenia hastata is readily eaten by stock.

Adenia as Human Poison

Wilful or accidental ingestion on non-edible (toxic) Adenia fruits, tubers, or medicine prepared from Adenia species has lead to human poisoning. Bryant, quoted by Watt et al. (1962), stated that a decoction of the root of Adenia gummifera is poisonous causing symptoms of vomiting and increased perspiration. Steyn (1941) noted that the tuber of Adenia glanca is non-poisonous but is easily confused with the poisonous ones. Green et al. (1923) reported that the Tswana used the fruit of Adenia digitata for homicidal purposes. They also stated that a small amount of fresh root of the plant was fatal to humans, causing severe gastroenteritis. Mettam (1932) reported that an Adenia had been

used for murder in the Meru district of Kenya.

The fruits of Adenia species which as noted above, resemble passion fruits are easily confused with edible fruits. Thus Verdoorn (1938) noted that the poisoning of fruits, tuber or medicines prepared from Adenia is always a possibility. Watt and Breyer (1962) reported a death of an eighteen year old girl that had eaten Adenia fruits.

Tha Akamba tribe of Kenya regard Adenia volkensii (Kiliambiti) as highly poisonous to human. Verdcourt and Trump (1969) reported that several cases of human poisoning including suicide had been recorded after ingestion of Adenia volkensii and that the plant was certainly poisonous. They related a documented case where a person poisoned by Adenia volkensii was attended by the Medical Officer, Kajiado Hospital, Kenya. They also reported on doubtful cases of the use of Adenia Schweinfurthii for criminal poisoning.

Adenia as Animal Poison

Adenia species have for quite sometime been incriminated to cause poisoning to stock. Green et al. (1923) stated that sheep and goats are susceptible to Adenia digitata poisoning. Steyn (1929) reported that a bulb of an Adenia species had proved to be rapidly fatal to an animal species which he did not identify in the record. The same author, in 1949, wrote that minced fresh leaves of Adenia glanca were not toxic to rabbits. Irvine, quoted by Watt et al. (1962), stated that slightly warmed stems and crushed leaves of Adenia are used as fish poison. He added that certain species were not as poisonous as described in the Onderstepoort report (1924). The Washamba use Adenia lobata as a fish poison. Adenia gummifera is also reported to be a fish poison,

(Verdcourt and Trump 1969). The same authors reported that Adenia volkensii is used in the Isiolo and Maralal areas as poison on bait for hyaenas.

The clinical symptoms and pathological changes, in animals and man, caused by Adenia poisoning have also been reported. Thus Green and Andrews (1923) noted that sheep and goats present symptoms of abdominal pain and purging after the ingestion of Adenia digitata. Steiner, quoted by Watt et al. (1962), reported that the bulb of an Adenia species, (So. Afr. Nat. Herb. No. 7611), proved to be rapidly fatal to an animal (species not specified) and that the symptoms were dypnoea and paralysis. He noted that the characteristic macroscopic findings of the internal organs were cyanosis and marked hyperaemia.

In human, Burtt (1926) reported that poisoning by watery extract of the tubers of Adenia digitata resulted in instant vomiting, prostration and probably some cardiac effect. In some cases, severe gastroenteritis also ensued. Verdcourt and Trump (1969) describing a case of Adenia volkensii poisoning at Kajiado, Kenya, noted that the symptoms were drowsiness and loss of tone of the muscle. The only macroscopic change observed was slight congestion of the liver. Watt et al. (1962) reported that the post-mortem lesions of two children dying after eating fruits of Adenia digitata were nephritis, haemorrhage in the liver, necrosis and vascular thrombosis of the large intestines especially rectum.

Chemical Research

Green and Andrew (1923) detected a cyanogenetic glycoside from the root of *Adenia digitata*. They found that the cyanogenetic glycoside was completely destroyed by dessication. The same authors also isolated a phytotoxin, toxalbumin modeccin. Steyn (1929) reported that the leaf of *Adenia glauca* contains hydrocyanic acid. He also said that the tuber does not contain any cyanogenetic glycoside. Githens, quoted by Watt et al. (1962), detected toxalbumin modeccin in the leaf and root of *Adenia globosa* and said that *Adenia kirkii* contains toxalbumin. Irvine, quoted by Watt et al. (1962), stated that the stem of *Adenia lobata* contains clear tasteless sap which turns red on standing and is used in arrow poisoning. Verdcourt and Trump. (1969) reported that no work had been carried out on the toxic principle of *Adenia volkensii* and that the allied South African species, *Adenia digitata* contains cyanogenetic glycoside and a toxalbumin modeccin.

PART THREE

EXPERIMENTAL STUDIES

PART III (a) SENECIO MOOREI TOXICOSIS IN ANIMALS

Experiment I. Rat

MATERIALS AND METHODS

Young branches with fresh leaves, flowers and shoots were collected from different areas of the Nyandarua District of the Central Province of Kenya and transported to the Faculty of Veterinary Medicine, Kabete. These were sundried and ground into fine powder in a standard No. 3 Willey Mill. The powder was used for toxicity studies in rats, bull calves and pigs.

Rats experiment was subdivided into two parts; part one dealt with acute and subacute Senecio poisoning, and part two chronic Senecio poisoning.

A group of hundred, one month old, albino rats was obtained from Kenyatta National Hospital, small animal unit. These were divided into two major groups of fifty rats each and were named Group A and Group B.

Experiment with rats in Group A was designed to determine whether rats would eat a large quantity of Senecio powder, to calculate the amount of senecio capable of causing poisoning and to study the clinical signs and the pathological changes of Senecio moorei poisoning.

The experiment with rats in Group B was intended to study the chronic poisoning of rats with Senecio powder as well as pathological changes in their chronic stages.

(i) Acute Senecio moorei Poisoning in Rats

Rats in Group A which were both males and females and between 49 and 70 Gm. in weight were divided into five groups of ten rats each and segregated by sex. These five groups were housed in separate all metal cages which were provided with saw-dust on the floor. Four of these groups were fed with senecio powder incorporated into a basal diet to make:-

50% first group

20% second group

10% third group

5% fourth group

The fifth group was used as control and fed with basal diet only. The basal diet consisted of commercially available chick mash on which an excellent growth of the control was obtained. Water was available all the time. The rats were observed daily, weighed twice a week and an average weight for one week recorded.

All rats that died naturally or were sacrificed were examined by standard necropsy procedure and sections taken from liver, kidneys, spleen, duodenum, stomach, large intestines, lungs, heart muscle and brain. The tissues collected were preserved in acetate buffered

10% formalin. Routine section were stained with haematoxylin and eosin. The histological and staining procedures followed were according to the Armed Forces Institute of Pathology Manual of Histologic Staining Methods (3rd Edition) 1968.

RESULTS

The rats that fed a diet containing 50% Senecio powder started dying on the 3rd day and on the 7th day only three remained which were sacrificed on the 8th day when in extremis. The second group of rats that fed a diet containing 20% Senecio powder started dying on the 5th day and on the 15th day only 2 remained which were also sacrificed. Rats that fed diets containing 10% and 5% Senecio powder were all dead between the 15th and the 45th day of the experiment. Natural death was frequent and there was a good deal of cannibalism particularly of rats dying during the night in the early stages of the experiment, so that few rats were not available for pathological or even post-mortem examination. TABLE I shows differences in weight and weekly mortality.

Clinical Findings.

Rats that died during the first two weeks of the experiment showed the following clinical signs.

For the first few days they fed poorly and lost some weight. The clinical symptoms were essentially very similar, starting with loss of appetite after which the animal showed increased weakness. The animal

though weak seemed to have some excitement, running about and climbing up the cage and making gnawing movements. No diarrhoeic stool was noticed. Respiration seemed to have been accelerated but it was difficult to determine how much this was. No convulsions were noticed. General weakness, staring coat yielded to deepening stupour, followed by coma; respiration slowed down and finally ceased.

The rats that died between the 15th and 45th day of the feeding experiment had lost much of their weight in comparison with the controls (TABLE I). Many rats were found in a state resembling coma, when they lay prostrate and felt cold. Their breathing was hardly perceptible though the heart continued to beat slowly for many hours. In some cases yellowish urine was noticed.

Macroscopic Lesions

At necropsy there was ascites and hydrothorax in rats that died during the first week of the experiment.

Liver: Livers were mostly enlarged, representing more than 5 per cent of the body-weight, against 3.5 - 4 per cent in normal controls. Sometimes they were mottled. Occasionally an animal had a small liver in which parts of one or more lobes were very pale or sharply separated from the darkly coloured part of the organ.

Spleen: The spleen varied considerably in size. Gastrointestinal Tract: The stomach of most rats was often distended with food which might be

black in parts, at other times the stomach and intestinal content was slimmy and the intestinal wall congested. Pancreas: There were no significant gross lesions observed on pancreas.

Rats that died between the second and fourth week of the experiment had also ascites and hydrothorax.

Liver: Livers were often smaller (about 3 per cent of the body weight) and mottled.

Lungs: These were sometimes congested or had petechial haemorrhages.

Spleen: In most cases the spleen was enlarged.

Brain: Hyperaemia of the brain was observed in more than 50% of the animals.

Kidney: The kidneys showed only slight congestion.

Heart muscle: The heart muscle was sometimes flabby.

Gastrointestinal Tract: Most of these animals had their stomachs distended with food.

Microscopic Lesions

Liver: The livers of most rats that died during the first two weeks of treatment were congested and there was haemorrhagic necrotic hepatitis. The haemorrhage was marked in the vicinity of the central vein from which the extravasation appeared to have originated (Fig. 4). Cases of sinusoidal congestion were also noted; necrosis of the parenchyma being most marked around the central vein, but frequently involoving two thirds of the liver lobule (Fig. 5). The necrosis of the hepatic cells was of

coagulative type whereby nuclei had completely disappeared or could only be discerned. Sometimes degenerate hepatic cells were seen scattered amongst areas of congestion and haemorrhage but frequnetly the hepatic cells had been replaced by haemorrhages in centrolobular areas.

At the peripheral zone of hepatic lobules there were apparent normal hepatic cells. In certain cases they formed a comparatively narrow zone; in others larger islands of cells were seen neighbouring necrotic tissue. These groups of cells did not always from a continous peripheral zone, but occurred mostly as islands around the portal tract. The cells at the periphery of these islands immediately adjacent to the necrotic cells showed slight vacuolation of fatty change. The cytoplasm was faintly granular and the nuclei were more or less normal in appearance. There were no mitotic figures seen.

Inspite of the variation in time of survival among the remaining rats their livers had many common histological features. The parenchymal damage was invariably present, the outstanding feature being the occurrence of large cells (megalocytes). The cells tended to occur in the periportal and mid-zones with more healthy parenchymal around the central vein, (Fig. 6). The nuclei of the big cells were round or oval, sometimes rather irregular in outline and usually had a sharply demarcated basophil nuclear membrane. The chromatin was rather scanty, and fragmented and the nucleali were large, often multiple and deeply

basophil. As well as nucleoli there were often some globules within these nuclei (Fig. 7). Evidence of hydropic degeneration, nuclear fragmentation and cytoplasmic vacuolation, often due to fatty change, as judged by Sudan IV stain were noticed in some of the cases examined (Fig. 8). In some sections there were spaces corresponding in size to a much enlarged parenchymal cell which contained cellular debris, often fatty, and sometimes nothing at all, (Fig. 9). There were neither parenchymal regeneration nodules nor bile duct proliferation observed in any one section.

Lungs: Most sections from the lungs showed congestion, sometimes haemorrhage or thickening of the alveolar wall. There were also cases of alveolar oedema. Murine pneumonia was noted in about 30% of the rats.

Kidneys: Two rats dying at the fourth week of the experiment showed marked depostion of hyaline casts within the convoluted tubule (Fig. 10) as well as vacuolation of the epithelial cells of the convoluted tubules.

Brain: Meningeal congestion and oedema were observed in two rats dying at later stages of the experiment.

Spleen: Apart from congestion and very slight fibrosis there were no other lesions seen in sections taken from spleen.

Gastrointestinal Tract: Slight occasional catarrhal to ulcerative enteritis was noted.

All other organs did not show any significant microscopic lesions.

Control rats killed after the 49th day of the experiment did not show either gross or microscopic lesions.

An estimation of the intake of senecio powder capable of causing death to a four week old albino rat was also carried out as follows:-

Five, four week-old male rats weighting between 55 and 65 Gm. were selected and kept separately in all metal cages. Each rat was fed with 20 Gm. of Senecio moorei powder mixed thoroughly with chicken mash in equal amounts. Wastage was reduced to minimum by feeding the animals separately with limited amount of mixture in a fixed container. Water was available ad libitum. Within the first three days of experiment all rats were dead. The exact amount of mixture consumed was calculated as in TABLE 2.

Total amount consumed was 15 Gm., average 3 Gm. consumed by each rat. An approximate amount of 1.5 Gm. Senecio moorei powder killed a rat in three days.

(ii) Chronic Senecio moorei Poisoning in Rats.

MATERIALS AND METHODS

In this experiment, it was intended to maintain the rats for a considerable time so as to study the chronic Senecio moorei poisoning in rats.

Animals in Group B mentioned above, consisted of fifty young locally bred albino rats weighing between 49 and 70 Gm. and both males and females.

These were subdivided into five groups of ten rats each and segragated by sex. Each group was housed as in previous experiment and were fed with Senecio moorei powder incorporated into basal diet, and thoroughly mixed, to make the following percentages:-

4%	-	first group
3%	-	second group
2%	-	third group
1%	-	fourth group
0%	-	fifth group

The fifth group of 5 male and 5 female rats was kept as control. The animals were weighed at approximately weekly intervals till death. Controls were kept under similar conditions as in previous experiment. Water and the feeding mixture were available ad libitum. The rats survived from 3 to 15 months of such treatment.

All the animals that died or were sacrificed were examined at post-mortem. The livers and other organs were fixed in acetate-buffered 10% formalin. Sections were stained with haematoxylin and eosin. Van Gieson stain was used for demonstration of connective tissue and periodic acid-Schiff's stain for glycogen.

RESULTS

Except for those rats that fed 4% Senecio powder, that lost some weight at the beginning of the experiment, there was no substantial difference in weight of the other groups and that of controls. Shortly

before death, however, the animals lost weight rapidly, developed yellowish, discoloured fur and sometimes a distended abdomen due to ascites. Rat No. KT 8 showed distended bladder due to obstruction of the urethra by wax-like concretions with accompanying overflow of urine.

The rats that fed 4% senecio powder started dying in the first week of experiment and were finished in the third month. Those rats that fed 3% and 2% senecio powder lived more than four months and were still alive in the 8th month. Rats that fed on 1% senecio powder lived for more than 14 months (TABLE 3).

Macroscopic Lesions

Liver: Animals that died within the first twelve weeks of the experiment (TABLE 3), did not reveal any significant gross liver changes. The livers were of normal shape and had smooth surface. Sometimes they were slightly diminished in size but often within the normal limits. The livers of those rats that survived between the third and the eighth month again showed macroscopic normal livers in respect to size, shape and smooth surface. The colour of most livers, however, was yellow to golden brown. Most animals that survived longer than 8 months showed slight increase in size of their livers. Three male rats showed macroscopic nodular hyperplasia of the livers, fairly uniformly distributed through out the organ (Figs. 11 and 12).

Lungs: More than 50% of the experimental animals had pneumonic lungs. Other organs appeared almost normal grossly.

Microscopic Lesions

Liver: The histological changes of the livers of rats that died within the first few weeks of treatment were characterized by congestion, haemorrhage and necrosis. Necrosis was sometimes seen to extend from periportal region to the mid-zone of the lobule. The hepatic cells sometimes exhibited an appreciable increase in size. The cytoplasm seemed well packed with finely granular material. The nuclei which were very variable in size showed an inconstant chromatin pattern which in some, was quite dense, and in others, open and vasicular with generally more than one nucleolus. There were no dilatation of the sinusoids seen nor was there any significant proliferation of the bile ducts.

The hepatic tissue of the rats that survived between the third and the eighth month was composed of varying proportions of enlarged cells showing the characteristics described above. Also seen were smaller regenerating cells diffusely distribted or crowded together without definate arrangement. Another characteristic feature seen here was diffuse endotheliosis spreading along the blood sinusoids as well in the central ans sublobular veins. These cells were rounded or polyhedral in shape and were developing into swollen spindle-shaped type resembling

flattened endothelial cells and fibroblasts. In certain cases these cells were merely lining cells swollen and more prominent than normal, but in the majority they showed active proliferation (Fig. 13) going on in certain instances to massive hyperplasia with partial or apparently complete obliteration of the lumen.

A third important feature seen in hepatic cells was the clear cytoplasmic vacuolation. In some cells these caused compression of the nucleus which gave an appearance of a dented spheroid (Fig. 14) or crescent (Fig. 15). The bile ducts were markedly prominent and in some places these showed pronounced hyperplastic change (Fig. 16).

Microscopic liver changes of 3 male rats which lived for more than 13 months were characterized by early trabecular hepatomas formation, much increase of bile duct formation, cystadenomas of the bile duct epithelium and the proliferation of fibroblastic tissue, (fibrosis) with subsequent formation of fibromas. Mitotic figures in these tumours were infrequent and there was no metastasis seen in any organ. The microscopic structure of these tumours was as described below:-

Bile-duct cystadenomas (Figs. 17 and 18). These tumours were histologically composed of grandular structures with their lumens filled with mucous coagulated exudate. The epithelium lining these glands was atrophied and in some cases completely desquamated. At times associated with these glands were wide bands of connective tissue which separated

the glands. These tumours were in some areas adjacent to a hepatoma.

Hepatomas (Figs. 19 and 20). Histologically the cellular pattern varied greatly. Some tumours had large cells while other tumours were composed of compressed cells smaller than normal liver cells but with an abnormal orientation into trabeculae. Where the trabeculae-like cells were seen, they appeared larger than normal. The width of the cellular column varied greatly. In some cases the hepatomas were associated with fibrosi An interesting feature was encapsulation of one hepatoma. All rats showe multiple tumours in the same organ.

Fibromas (Fig. 21). The characteristic microscopic picture seen here consisted of whorls and interlacing bundles of fibroblasts and collagen fibres. The cells were spindle shaped with pale elongated nuclei.

Control rats, all of which survived for 15 months showed no areas of hyperplasia or any histological changes in the liver.

Brain: Rat No. KT 7 which died at the 14th month of experiment showed extensive cerebellar haemorrhage (Fig. 22). There were also areas of extravasation in the cerebral tissue.

Lungs: Chronic murine pneumonia and consolidation of parenchyma were seen in more than 50% of the experimental rats.

Peritoneum: In four rats ascities ranging between 10 and 30 ml. was present.

Kidneys: The kidneys of three rats dying after the 10th month showed lesions ranging from degenerative changes of the epithelial cells of proximal convoluted tubules to necrosis of the tubules (Fig. 23).

Experiment II. Bovine

MATERIALS AND METHODS

Four bull calves between 8 and 12 months of age and weighing between 97 and 120 Kg. were obtained from the Veterinary Research Laboratory, Kabete. They were maintained at the Faculty of Veterinary Medicine, Large Animals Unit. Two bull calves were assigned to an experimental group to be drenched 400 and 200 Gm. Senecio powder suspended in 1 litre and 800 ml. tap water respectively, using bottle. The other two bull calves were kept as controls. All animals received a basal diet of hay and a grain ration daily. Body temperatures were taken daily. The animals were also weighed weekly.

Collection of Blood Samples and Determination of Blood Constituents

All samples for haematological and biochemical analysis were collected daily in the morning around 9. a.m. They were taken from jugular vein using 2" 14 gauge needles and collected in bijou and universal bottles. For unclotted blood dried disodium ethylene-diaminetetracetic acid (EDTA) was used as anticoagulant at a concentration of 1-2 mg./ml. blood.

To make the anticoagulant a 10% (W/V), solution of EDTA was made up in distilled water and then 0.1-0.2 ml. of this solution was put into bijou bottles. The bottles were then allowed to dry at room temperature. About 3-4 ml. of blood was collected in this way being gently shaken to allow the blood and the anticoagulant to mix.

For determination of serum enzymes, about 20 ml. of blood were collected in universal bottles containing no anticoagulant. The bottles were allowed to stand for a while in an incubator and then centrifuged at room temperature for 30 min. at 2000 r.p.m. in an M.S.E. (Measuring Scientific Equipment) centrifuge. The serum was then pipetted off and used for Protein and serum Transaminase determination.

(1) ESTIMATION OF BLOOD VALUES

(a) The Erythrocytes and Leukocytes Count, Mean Corpascular Volume and Haemoglobin determinations.

The electronic counting technique using Coulter Counter (Coulter Electronics Inc., Hialeah, Florida) was employed.

After mixing the blood thoroughly a dilution of 1:500 was made for W.B.C. Count and HB determination using isoton. From this dilution a further 1:50,000 dilution was made for R.B.C. and M. C. V. determinations. Using Coulter Counter Model Z B, R.B.C. Count and M.C.V. determination were done at Amplification Setting of 1 Aperture Current of .354 and Threshhold of 10. For R.B.C. the first 3 figures were corrected from the Coicindence Correction Chart and results expressed in million cells/ml. The M.C.V. was calculated as follows:-

$$MCV = \frac{PCV \times 10}{RBC}$$

To the 1:500 dilution 6 drops of Zap-globin were added and mixed to haemolyze the R.B.C. The W.B.C. count was done on the above Setting,

except Threshhold which in this case was 20. Any figure beyond 10,000 was corrected from the Coicindence Correction Chart and the results expressed in thousand of cells/ml. The remainder of this dilution was poured into the haemoglobinometer and the HB in gm./100 ml. of blood was read directly.

(b) Differential Leukocyte Counts

These were done using the technique described by Dacie and Lewis (1968). Blood films were made normally and then stained with Giemsa stain for 30 min. Then through examination in a strip running the whole length of the film at least 200 leukocytes lying along this strip were counted. The numbers of lymphocytes, neutrophils, eosinophils etc. counted in this manner were then expressed as percentage of the total number of cells counted.

(c) The Packed Cell Volume

The microhaematocrit method described by Dacie and Lewis (1968) was used. Commercially available unheparinized (plain) microhaematocrit capillary tubes,(Arthur H. Thomas Co., Philadelphia 5, USA) 75 mm. in length and with an internal diameter of 1.3-1.5 mm. were used. The tubes were filled with the uncoagulated blood by capillary action until about three quarters of each tube was full of blood. The dry end of the tubes were then sealed by heating in bunsen flame. The tubes were spun at 12,000 r.p.m. for 5 min. in a microhaematocrit centrifuge (Hawksley and Sons Ltd. London). Dacie and Lewis (1968) quoting Garby and Vuille (1961)

estimate the amount of trapped plasma at 1.1-1.5% (mean 1.3%). The percentage packed cell volume was determined from the scale of a Hawksley microhaematocrit reader. The buffy coat layer was not included in the reading.

(2) SERUM DETERMINATIONS

(a) Determination of Serum Glutamic Oxalacetic Transaminase (S.G.O.T.) and Serum Glutamic Pyruvic Transaminase (S.G.P.T.) Values.

This was done following the method of Reitman and Frankel (1957) as outlined in Sigma Chemical Company's 1963 Technical Bulletin No. 505. The activities of S G O T and S G P T were estimated in a "Spectronic 20" (Bausch & Lomb, Inc, Rochester 2, N.Y. U.S.A.) at a wavelength of 505 mu and the results were expressed in Sigma-Frankel (S-F) Units.

(b) Determination of Serum Alkaline Phosphatase (A.P.)

The technique employed was that of Kind and King (1954) using 4-amino-antipyrine. Serum was incubated at 37^{0} for 15 minutes with phenyl phosphate solution at pH 10.0. The amount of phenol liberated was estimated colorimetrically using 4-amino-antipyrine which yields a red coloured quinone. The results obtained were expressed in King-Armstrong (K-A) Units.

(c) Determination of Total Protein (T.P.) Concentration and Albumin to Globulin (A/G) Ratio

This was done by the "Improved" Biuret method of Weichselbaum (1946) as outlined in the "Lab-Trol Manual" (Dade Reagents Inc., Miami, Florida, USA). The activities of the Total Protein and of Albumin were estimated in a "Spectronic 20" (Bausch & Lomb, Inc., Rochester 2, N.Y. USA) at wavelength 540 mu.

Calculation

$$\% \text{ T.P. value of Unknown} = \frac{\text{O.D. of Unknown}}{\text{O.D. of Standard}} \times \text{T.P. Value of Standard}$$

$$\% \text{ Unknown} = \frac{\text{O.D. Albumin in Unknown}}{\text{O.D. of Standard T.P.}} \times \text{Standard T.P. Value}$$

$$\% \text{ Serum Globulin} = \text{T.P.} - \text{Albumin value}$$

$$\text{Albumin/Globulin Ratio} = \frac{\%\text{Albumin}}{\%\text{Globulin}}$$

Standard necropsy was performed on both experimental and control animals. At necropsy the tissue were collected and prepared for histopathological examination in the following general manner: the sections were selected from all macroscopic lesions - where these were seen - and routinely from the following organs: brain, liver, heart pancreas, kidney, lungs, stomach, duodenum and jejunum. They were preserved in acetate buffered, 10% formalin. Routine sections were stained with haematoxylin and eosin. Van Gieson Stain was used for selected materials to demonstrate collagen fibres. Again here the histological and staining procedures

followed were according to the Armed Forces Institute of Pathology Manual of Histologic Staining Methods (3rd Edition) 1968.

RESULTS

Bull calf No. 6688 died after 91 days and No. 6540 was sacrificed in moribund state after 112 days of the experiment. The experimental data on weight, SGOT, SGPT, Serum Protein, A.P. and Blood picture are summerized in TABLE 4 and Appendix and respectively.

There was a steady rise of SGOT in experimental animals especially from the 11th week until death. No significant difference in either the SGPT or AP was noticed between the experimental animals and the controls although individual variation was there. The serum proteins showed a slight fall in albumin and slight rise in globulin. The total protein, however, remained unchanged. Daily temperatures were constant throughout the experimental period except for a slight fall before death in experimental animals. Except for individual variations, blood picture showed no difference between the experimental and control animals. Progressive loss of weight was noticed in experimental animals especially after the first month of drenching.

Clinical Findings

Immediately before death the animals were very dull and tended to lie down; were not eating but appeared full. Bull calf No. 6688 showed

slight scouring and weakness of the hind-quarters. Sometimes the animals were noticed shivering. They looked emanciated but did not show any marked evidence of jaundice in any of the mucous membranes.

Macroscopic Lesions

Both animals had serous exudate in thoracic and abdominal cavities (Fig. 24) varying between 400 c.c. and 2,000 c.c. Bull calf No. 6688 had also some exudate in the pericardial sac (Fig. 25). In all cases lungs looked congested. There were ecchymotic haemorrhages on the epicardium, endocardium, pleura, diaphragm and the gall bladder. The gastrointestinal mucosa was congested. Bull calf No. 6688 showed meningeal congestion (Fig. 26). There was some thickening and petechiation of the abomasum. The kidneys, spleen and urinary bladder appeared grossly normal. In one case the liver was enlarged, swollen and had a thickened opague capsule without nodularity (Fig. 27) and in the other it was fibrotic, small and yellowish in colour. There was no marked evidence of jaundice in any other organ.. In one case the gall-bladder was enlarged (Fig. 27) with submucous and subserous exudates and was about 0.5 inch thick, but the contents appeared normal.

Microscopic Lesions

The histological findings of the liver, kidneys and lungs in these two bull calves were almost similar and the lesions may be summarized as follows:

Liver

Fibrosis: Marked degree of fibrosis particularly in the portal triad as well as around the central veins was evident in almost all section observed. This consisted of new collagen formation (Figs. 28 and 29); lose and extensive in some sections but slight in others. The new collagen was usually in the form of fine threads and non-cellular, but more cellular in areas of new bile duct formation. In some parts interlobular fibrosis was extensive and often pericellular, thus rendering the lobular arrangment less clear (Fig. 30). Fibrosis apparently resulted as a replacement following the death and disappearance of hepatocytes and even the remaining parenchymatous cells were strangled by this fibrous tissue. Some sections showed areas of marked fibrosis, haemorrhage, bile-duct proliferation and plenty of inflammatory cells composed of neutrophils as well as eosinophils. Also seen were fine reticulin fibres infiltrating among the healthy parenchymal cells.

Bile-duct proliferation: This was well marked in a number of sections but entirely absent in others. They appeared to grow in the vicinity of the portal tract and radiated in all directions. Proliferation of these ducts occurred in form of tubular structure in a few instances very pronounced and adenomatous in character as well as diffuse biliary cells without duct formation (Figs. 31 and 32).

Regeneration of liver cells: Some sections showed regenrating liver cells in form small scattered islets(colonies). with nuclei which

were much smaller than those of the older surrounding liver cells.

Atrophy: Some lobules were reduced in size with reduced number of cells on microscopic examination.

Megalocytosis: In areas where the lobules although atrophic remained distinct, the hepatocytes appeared larger than normal (megalocyte and a number of nuclei were already pyknotic. The general picture, however, was not that of megalocytosis.

Occlusion of hepatic vein (V.O.D.) : This was particularly characterized by persistent fibrosis in the centrolobular (non-portal) areas. The vessels, some of which had thickened walls, had much diminished lumen (Fig. 33).

Lungs: In both animals the lesions were where characterized by intense emphysema, haemorrhage and oedema of the lung tissue (Fig. 34).

Kidneys: These also showed intense congestion of the capillaries particularly those of the medullary portion their diameter being equal to that of neighbouring collecting tibules.

Brain: One animal showed meningeal congestion and oedema.

Experiment III. Swine

MATERIALS AND METHODS

Senecio powder that had previously been shown to produce fatal toxic effects in rats and cattle was again used with the intention of studying its toxic effects in this animal species. Six Large-White pigs weighing approximately 24 Kg. and about 8 weeks of age were used.

They were divided into 3 groups of 2 pigs each. A well balanced, growing type swine ration (Sow and Weaner Meal) commercially obtainable was fed. Two sets of experiments were designed.

Experiment III (a), with two pigs in group I, was intended to determine whether pigs would eat a ration containing high concentrations of Senecio, whether any disease would ensue and to study the pathological changes. These pigs fed 25% Senecio powder incorporated into a basal ration (Sow and Weaner Meal). The rations were fed on an ad libitum basis for 60 days and fresh water was supplied all the time. No controls were kept. The pigs were observed daily. At the end of 60 days of treatment both pigs were sacrificed by an intravenous injection of Pentobarbitone Sodium followed by exanguination. They were necropsied and selected tissues collected for histopathological examination.

Experiment III (b), with pigs in group II was designed to study closely the syndromes of senecio poisoning in pigs using a comparatively low concentration of senecio powder in the ration. The two pigs fed 20% senecio powder incorporated into a basal diet. They were again fed on ad libitum basis for 75 and 90 days respectively and water was supplied continously. The pigs in group III were kept as controls and fed on basal diet only.

In the second experiment, temperatures were taken daily, the food remaining was weighed weekly and the pigs were weighed weekly and at death. Blood samples were taken immediately before death. Animals were killed by intravenous injection of Pentobarbitone Sodium followed by exanguination.

Samples of serum and unclotted blood were taken, EDTA being used as anticoagulant. Serum samples were examined for alkaline phosphatase by the method of Kind and King (1954) and results obtained expressed in King-Armstrong units. Serum protein values were determined, by the "Improved" Biuret method of Weichselbaum (1946). Unclotted blood was examined for total red cells count, total and differential white cells count, erythrocytes sedimentation rate, haemoglobin and copper content.

Samples of liver and kidney were also analysed for copper content. Estimation of copper in blood, liver and kidney was by method similar to those of Clare et al. (1945) and Edenad and Green (1940).

Necropsies were carried out on all animals killed after certain intervals of experiment. Tissues collected from most organs were fixed in buffered 10% formalin embedded in paraffin wax, sectioned at 5 - 7 u and stained with haematoxylin and eosin. Selected sections from liver were also stained by Van Gieson stain for collagen. Some sections were fixed in Carnoy's fixative and stained with Best's Carmine stain for glycogen.

RESULTS

Experiment III (a)

Ration containing 25% *Senecio moorei* was eaten eagerly in the first two weeks but thereafter the pigs fed poorly. Both pigs grew

poorly and at the end of 60th day they were sacrificed when in apparent good health. Macroscopically there was no significant abnormalities of the internal organs. The livers were normal in colour and their sizes within the presumed normal range of their ages.

Microscopic Lesions

Liver:

Sections from livers of both pigs showed slightly thickening of the capsule (Fig 35) and slight increase of the bile duct cells. A number of the parenchymal cells in the periphery of the lobules had enlarged nuclei and there were few foci of parenchymal necrosis infiltrated with lymphoid cells. There was increased glycogen deposition in the cytoplasm of the parenchymal cells as judged by Best's Carmine stain. None of these livers showed evidence of fibrosis, steatosis or ductule proliferation.

Lungs:

Some sections showed extensive congestion, slight oedema and thickening of the alveolar walls (Fig. 36),. Very slight alveolar epithelialisation was noticed in one section, (Fig. 37).

Kidneys:

The only lesion observed here was the enlargement of some nuclei of the proximal convoluted tubules. These nuclei were darker than normal and sometimes cells containing these nuclei showed evidence of increased cytoplasm.

Other organs did not reveal any significant microscopic lesions.

Experiment III (b)

For the first three weeks the pigs in group II fed relatively well but thereafter lost appetite and started feeding poorly. Fig No. S 97 was sacrificed after 75 days of treatment while still in apparent good health. One control was also sacrificed. After 15 days the other experimental pig and control were sacrificed; again in apparent good health.

The weight gains during the experimental period and the weights of selected organs at the time the pigs were sacrificed and necropsied are given in TABLE 5. TABLE 6 summarizes blood picture, sedimentation rate, total protein, haemoglobin and copper content values. The principal points of interest were a decreased average weight gain in the experimental animals as compared with the controls, a relative neutrophilia in the blood samples from pigs that fed Senecio moorei material and slight rise of total serum protein levels in experimental pigs. Other findings were within the normal range for animals of this age.

Macroscopic Lesions

Except the spleen and lungs from pig No. 79 all other internal organs from both pigs did not show any significant abnormality. Pig. No. 79 showed the following lesions:-

Lungs: There was a widerspread subpleural and interstitial emphysema. The organ looked purple in colour with scattered red patches.

Spleen: This was very flabby and congested. On cut surface a considerable amount of blood oozed out of the organ.

No gross lesions were seen in control pigs.

Microscopic Lesions

Liver: The pig killed at 75 days of the experiment showed moderate enlargement of peripheral parenchymal cells. No area of necrosis of the parenchymal cells was noted in any of the sections observed. The pig that died at 90 days of the experiment showed almost similar lesion but slightly advanced (Figs.38 and 39).

Kidney: Both the pig that died after 75 days and that died after 90 days showed karyomegaly of the cells of proximal convoluted tubules.

Lungs: Pig No. 97 showed slightly epithelialized alveoli, there were areas of emphysema, thickening of the alveolar walls and congestion. Pig No. 79 showed almost similar lesions although slightly advanced (Fig. 40).

Spleen: Pig No. 79 showed a microscopically very haemorrhagic spleen. The white and red pulp had been almost replaced by haemorrhages. Other organs did not reveal any significant microscopic lesions. Sections taken from various organs of the control animals did not reveal any microscopic lesions.

DISCUSSION FOR PART III (a)

The acute hepatotoxic actions of the pyrrolizidine (Senecio) alkaloids have been reviewed by Chen (1945), Schoental (1961) Magee (1964) and Bull et al. (1968). The typical reactions of small laboratory animals to doses close to the LD 50 is a confluent, haemorrhagic and centrilobular necrosis in the liver. In general, the same parttern follows dosing by mouth, subcutaneously or intraperitoneally. Reactions following this parttern have been described for cats and rabbits (Cushny 1911), hamsters (Harris et al. 1957), mice (Chen et al. 1940) chicken (Campbell 1956) and rats (Barnes et al. 1964, Davidson 1935 and Selzer et al. 1951). Smaller doses give rise to subacute liver lesion characterised by the appearance of enlarged liver cells, a syndrome termed megalocytosis (Bull 1955).

In the present studies rats, cattle and pigs were used. In the first rat experiment, varying dosages comprising of 50%, 20%, 10% and 5% Senecio material were used. Constant correlation between survival time and dosage was established. The first group of rats that fed 50% Senecio material died within the first seven days of the experiment while the fourth group that fed 5% Senecio material were not all dead until the 7th week. The acute and subacute liver lesions observed in these rats were similar to those described by earlier workers such as Davidson (1935).

Apart from liver changes, lesions were also encountered in lungs, kidneys, brain and the gastrointestinal tract. Experiments specifically

designed to investigate the action of Senecio alkaloids in lungs have been reported by Lalich and Merkow (1961), Kay and Heath (1970) and Barnes et al. (1964). A survey of literature, however, indicates that there has only been passing references to lesions in brain and kidneys. These organs, particularly kidney, which is a candidate for metabolic activation of the alkaloids, should be investigated.

In the second rat experiment the dosage was adjusted to avoid acute toxic effects with high mortality within the first 3 months and the rats were maintained in apparently good health for periods of 3 to 15 months. Nevertheless these rats developed extensive degenerative lesions accompanied or followed by regeneration, hyperplasia and in some cases by neoplasia of the liver parenchyma. The range of the pathological changes observed in these rats was similar to that already described by Cook, Duffy and Schoental (1950) and Schoental, Head and Peacock (1954) using S. jacobaea.

In most of the experiments that produced tumours, alkaloid dosing was interrupted or ceased altogether for several months before death. In the present work the feeding of Senecio moorei continued uninterrupted until death. Three male rats treated with 1% Senecio powder developed tumours. Perhaps the small dosage here may help to explain why it was not necessary to interrupt the treatment.

As with most other hepatotrophic carcinogens, the female rats were less susceptible than the males to similar dosage of S. moorei.

Similar findings have been reported by Schoental et al. (1954) using Senecio jacobaea and retrorsine. The mechanism of action of Senecio alkaloids is not yet known (Schoental et al. 1954). It remians to be shown whether the hepatotoxic action is due to the parent alkaloids present in the Senecio moorei material or to any of their metabolic products.

In cattle, the terminal lesions in the two experimental animals were very similar. The most significant lesions were portal fibrosis with invasion of the lobule, bile-duct proliferation, regeneration of the liver cells, atrophy and veno-occlusive lesions (VOD) of the central and hepatic veins.

The extent and severity of these perenchymal, stromal and vascular lesions increased with longer survival on drenching. However, the gross and histologic appearances of the liver of the longer surviving calf No. 6540 were dominated by massive fibrosis distorting the lobular pattern and replacing much of the perenchyma, resembling classical Senecio "Cirrhosis" (Craig et al. 1930).

The sequence of events in the development of VOD were described by Bras and Hill (1956). Fibrosis in the walls of central and hepatic veins in field cases of Senecio poisoning in cattle and horses was seen by Craig et al. (1930). Subsequently there have been numerous reports of VOD in domestic and laboratory animals following the natural and

experimental intake of various pyrrolizidine alkaloids, for example Berry and Bras (1957), Hill and Martin (1958), Markson (1960), Schoental and Magee (1957). The lesions seen in the present calves were not dissimilar to those described in literature. The term "megalocytosis" was used by Bull (1955) to describe the effect of hepatotoxic pyrrolizidine alkaloid on the liver cells of animals. Similar cellular changes have been described as a result of various other hepatotoxic agents such as aflatoxin (Allcroft, Carnaghan, Sargeant and O'Kelly 1961) and thioacetamide (Wachstein, Meisel and Falcon, 1962). In the present study there was little evidence of megalocytosis.

The term pericellular cirrhosis was used by Craig et al. (1930) to describe connective tissue lesions in spontaneous ragwort poisoning, but later workers (Hill 1960, Markson, 1960) have preferred the term hepatic or portal fibrosis. Bull (1955) considered that the fibrosis was due to replacement of degenerate cells whereas Hill (1960) described fibrosis as an integral part of the response to Senecio alkaloids. The findings in the present calves support this later view as apart from the replacement of degenerate hepatocytes by fibrous tissue, fine reticulin fibres were seen infiltrating among the remaining perenchymal cells and along sinusoids.

Ford and Ritchie (1968) made the first detailed study of serum changes following the feeding of Senecio jacobaea to calves. They found

an increase in the concentration of S.G.O.T., fall in serum albumin and rise in serum globulin. The present work support their findings with exception that here the rise of S.G.O.T. was constant until death.

Harding et al. (1964) reported for the first time the extrahepatic lesions in the lungs and kidneys due to pyrrolizidine alkaloidosis in pigs.

The pigs used in their experiment were of the Landrace-Wessex Strain. Similar experiments but using pigs of the Large White Strain were reported by Bull et al. (1968). They were, however, unsucoessful in producing the advanced lesion in the lungs but the lesion in the kidney was identical with that produced by Harding. Possibly the difference may have been due in part to the difference in strain of the pigs (Bull et al. 1968). In the present studies pigs of the Large White Strain fed on a diet containing dried ground Senecio moorei. The results obtained were identical to those reported by Bull et al. (1968). The results with Senecio poisoning in pigs seem to illustrate an outstanding difference in species of animals in their reaction to the intoxication by pyrrolizidine alkaloids of these plants.

TABLE 1

Weight Changes (in Gm.) in Rats Fed on Varying Percentages of Senecio Powder

Duration in days	Percentage					Number of rats alive
	50	20	10	5	0 (Control)	
1	619	678	651	598	621	50
7	162	339	661	578	651	36
14	-	80	552	610	728	32
21	-	-	389	551	760	25
28	-	-	218	411	777	21
35	-	-	67	298	801	15
42	-	-	-	134	855	12
49	-	-	-	-	896	10

TABLE 2

Amount of Senecio Powder Mixture Consumed by Each Rat

During the Experimental Period

Rat	Weight in Gm.	Amount of mixture in Gm.	Amt. consumed in Gm.	Remainder in Gm.
1	57	20	3	17
2	55	20	3	17
3	59	20	2	18
4	62	20	4	16
5	65	20	3	17

TABLE 3

Mortality in Rats Poisoned With Low Concentration of Senecio Powder in the Diets

Sex	Percentage	Duration in months														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
M&F	4	4	2	4*												
F	3	3+	2	2	1*	2										*
M	2	1	3	2	-	-	1	1	2*							
M&F	1	-	-	1+	-	-	1*	1*	1	-	-	-	2*	1*	2	1
M&F(C)	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Sacrificed (mainly in extremis). F (Female) M (Male) C (Control)

\+ Canibalism. - No rat died.

TABLE 4

Body Weight Changes (in Kg.) and Total Weekly Amounts of Senecio Powder Consumed by Bull Calves.

Week	No. 6540	No. 6688	No. 6686	No. 6580	Total Weekly Amount of Senecio in Gm.
1	102	94	76	120	4,200
2	108	93	74	118	4,200
3	108	92	74	120	4,200
4	106	89	68	116	4,200
5	106	84	70	120	4,200
6	110	84	68	120	4,200
7	105	72	74	126	4,200
8	101	80	78	130	4,200
9	98	84	80	136	4,200
10	98	82	80	136	4,200
11	95	80	84	138	4,200
12	96	80	84	140	4,200
13	90	76	88	143	4,200
14	85	-	89	148	1,400
15	81	-	94	152	1,400
16	79	-	99	153	1,400

TABLE 5

Body Weight Changes, Liver, Kidney and Spleen Weights of Pig Fed 20% Senecio Powder.

Pig No.	S 66 M*	S 97 F**	S 76 F	S 79 M
Treatment	None	S.Moorei	None	S. Moorei
Days on Experiment	75	75	90	90
Initial Weight (Kg.)	23.2	21.8	23.6	26.4
Final Weight (Kg.)	67.3	41.4	71.8	45.9
Average daily gain (Kg.)	0.59	0.26	0.54	0.22
Liver Weight (Gm.)	1298	1165	1302	1058
Kidney Weight (Gm.)	110	102	118	98
Spleen Weight (Gm.)	112	114	152	68

* = Male

** = Female

TABLE 6

Blood, Alkaline Phosphatase (A.P.), Total Protein and Copper Values in Pigs Fed 20% Senecio Powder

Pig Number	Day on Experiment	R.B.C. Sedimentation Rate, mm/hr.	W.B.C. x 10^3	R.B.C. x 10^6	N%	B%	E%	L%	M%	HB Gm/100ml.	A.P. K-A Units	Total Protein Gm/100ml.	Coppes values		
													Blood mg/100 ml.	Liver (p.p.m./DM)	Kidney (p.p.m./DM)
S 66*	75	2	13.0	7.1	18	0	2	68	12	11.7	15	6.7	0.12	22	30
S 97	75	4	16.9	6.92	54	0	2	52	4	12.6	14	8.0	0.13	17	31
S 76*	90	5	12.9	6.77	28	0	1	55	9	12.2	10	5.8	0.11	24	29
S 79	90	6	17.4	6.51	50	0	3	39	5	13.0	16	5.4	0.13	24	35

*Controls

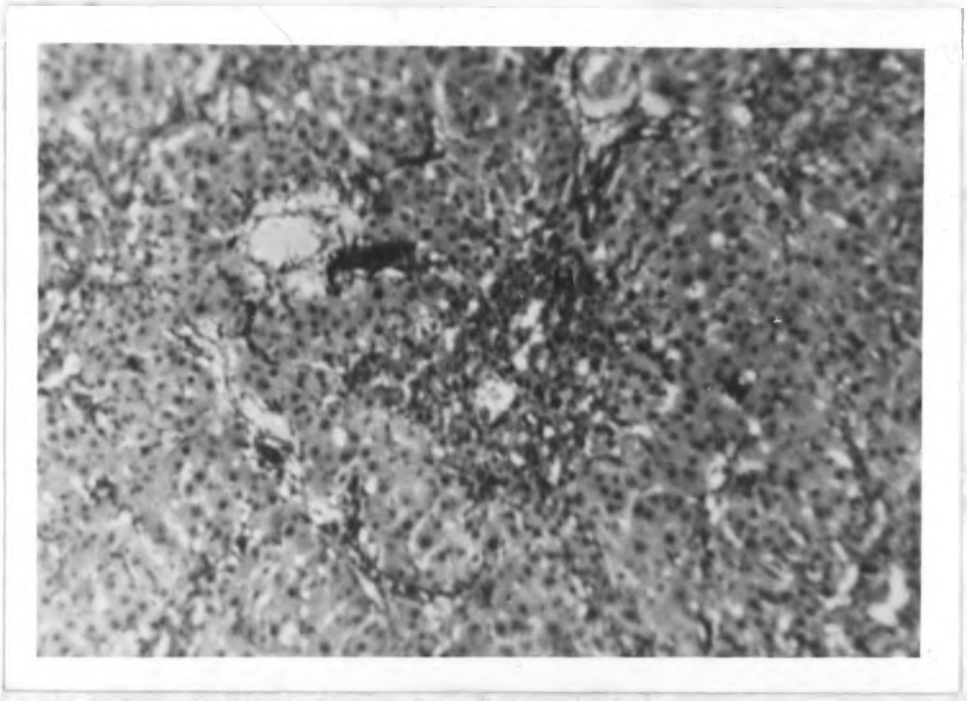

Fig. 4. Centrolobular haemorrhage in the liver of rat fed 20% Senecio powder for 13 days. H&E Stain: X 156.

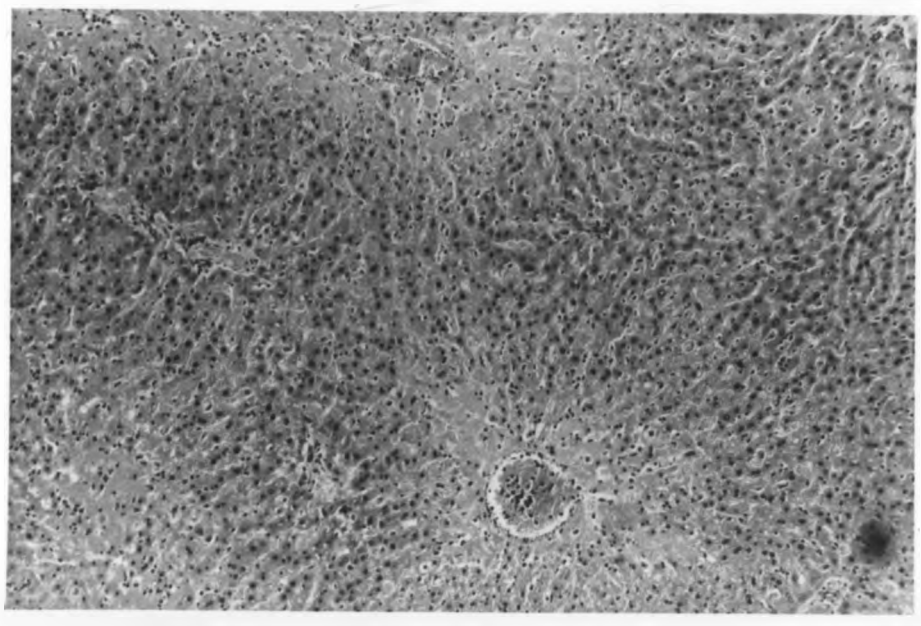

Fig. 5. Centrolobular necrosis in the liver of rat fed 10% Senecio powder for 28 days. H&E Stain: X 156.

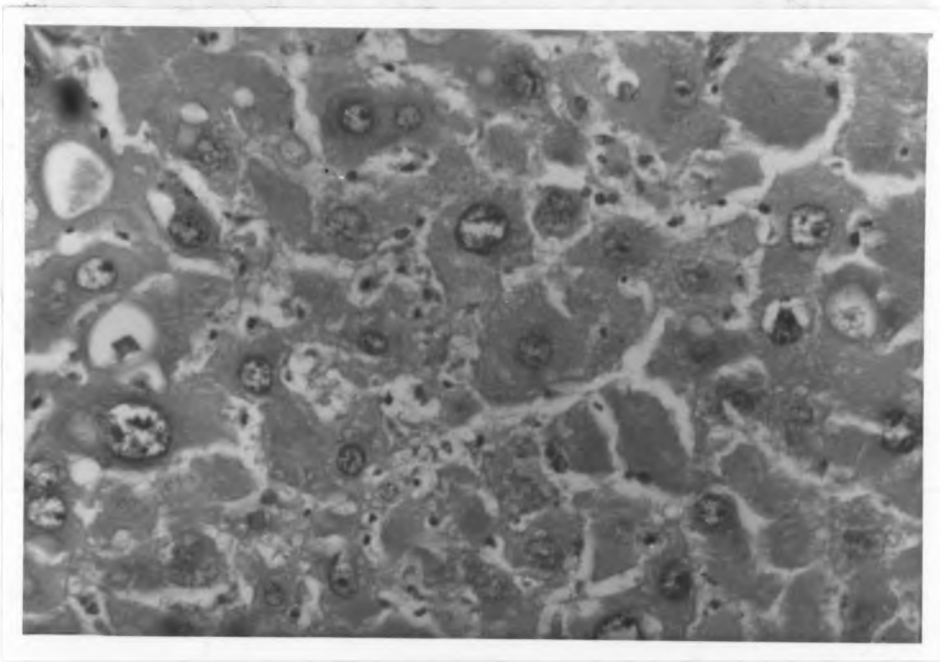

Fig. 6. Large hepatic cells (megalocytes). Liver section of rat fed Senecio powder for 44 days. H&E Stain:X391.

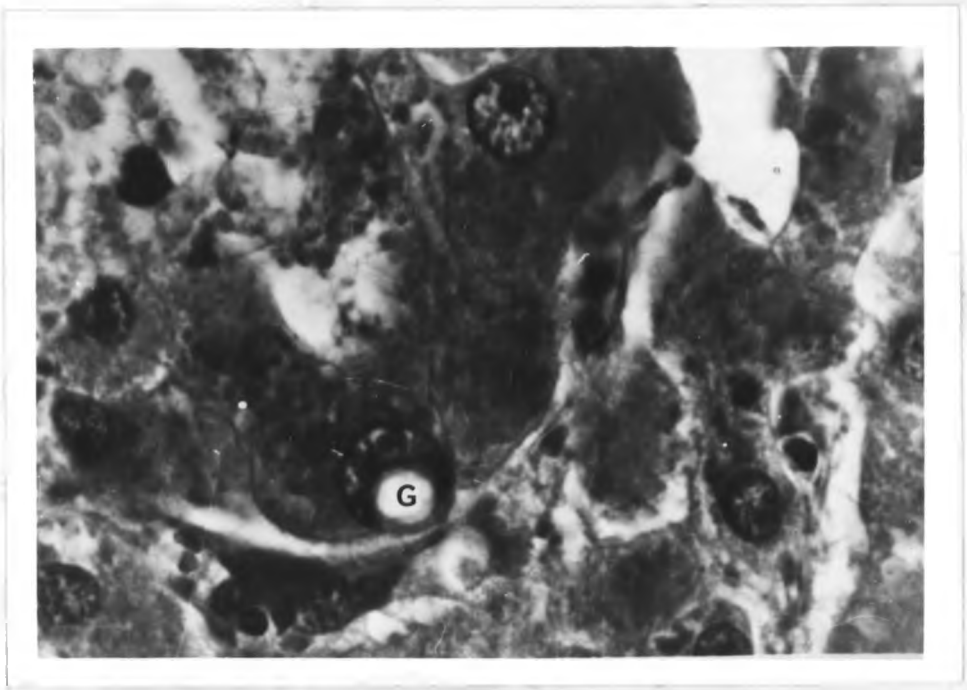

Fig. 7. A large globule (G) in the nuclei of hepatic cell of a rat fed 10% Senecio powder for 38 days. H&E Stain: X 625.

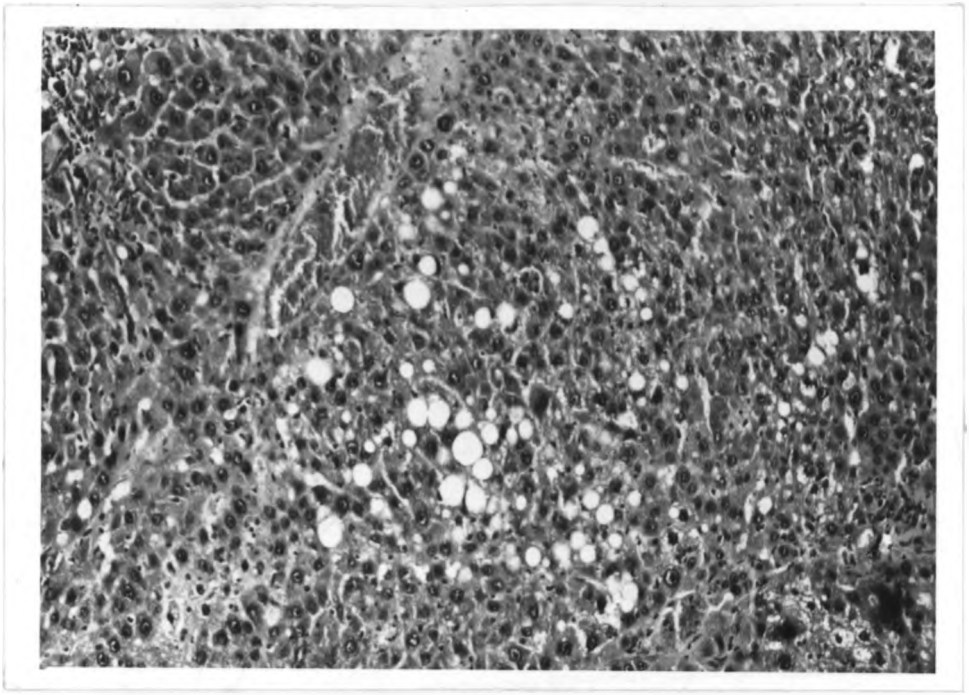

Fig. 8. Cytoplasmic vacuolation. Fatty degeneration in the liver of a rat fed 20% Senecio powder for 15 days. H&E Stain: X 156.

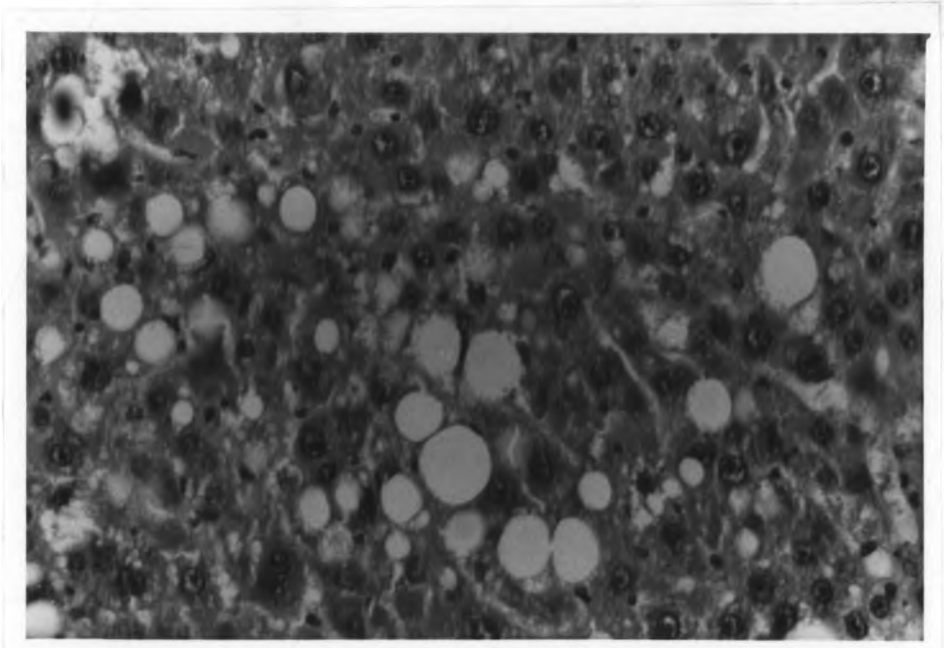

Fig. 9. Empty spaces corresponding in size to enlarged parenchymal cells, containing nothing. Higher power of Fig. 8. H&E Stain: 391.

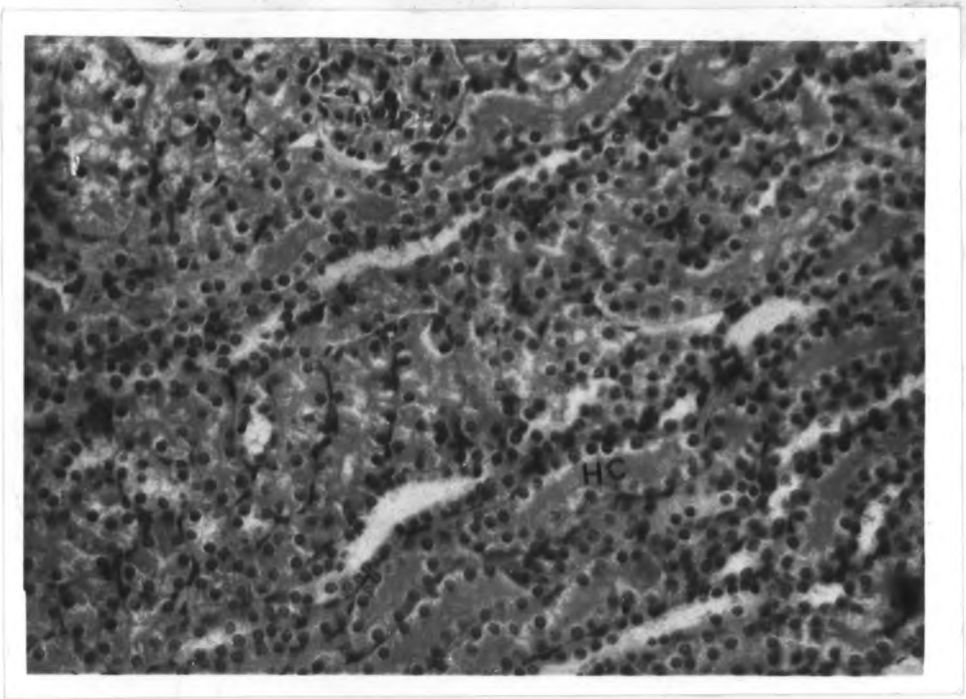

Fig. 10. Hyaline casts (HC) in the convoluted tubules of kidney of a rat fed 5% powder for 44 days. H&E Stain: X 391.

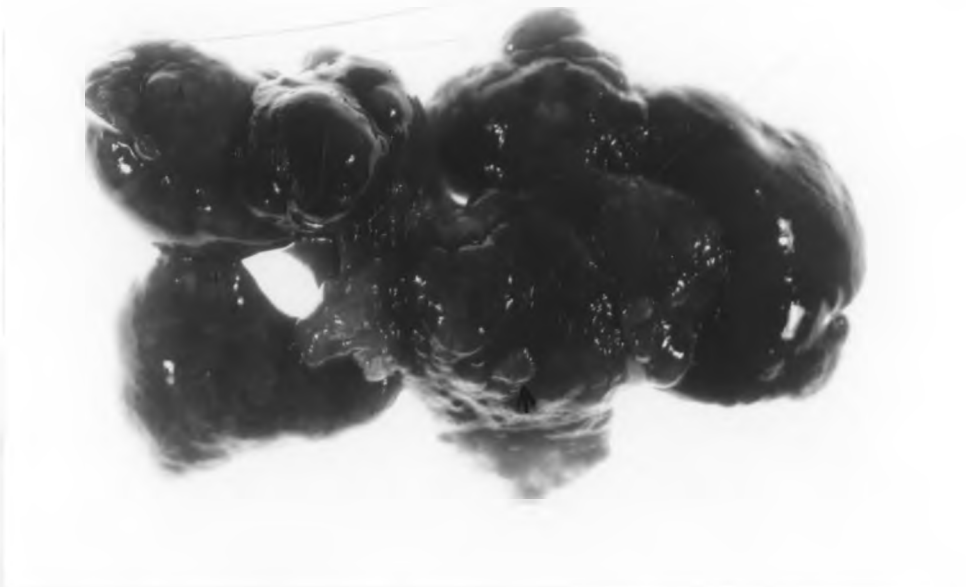

Fig. 11. Liver of a rat that had been fed 1% Senecio powder for 13 months. Arrow shows a hyporplastic nodule of the liver.

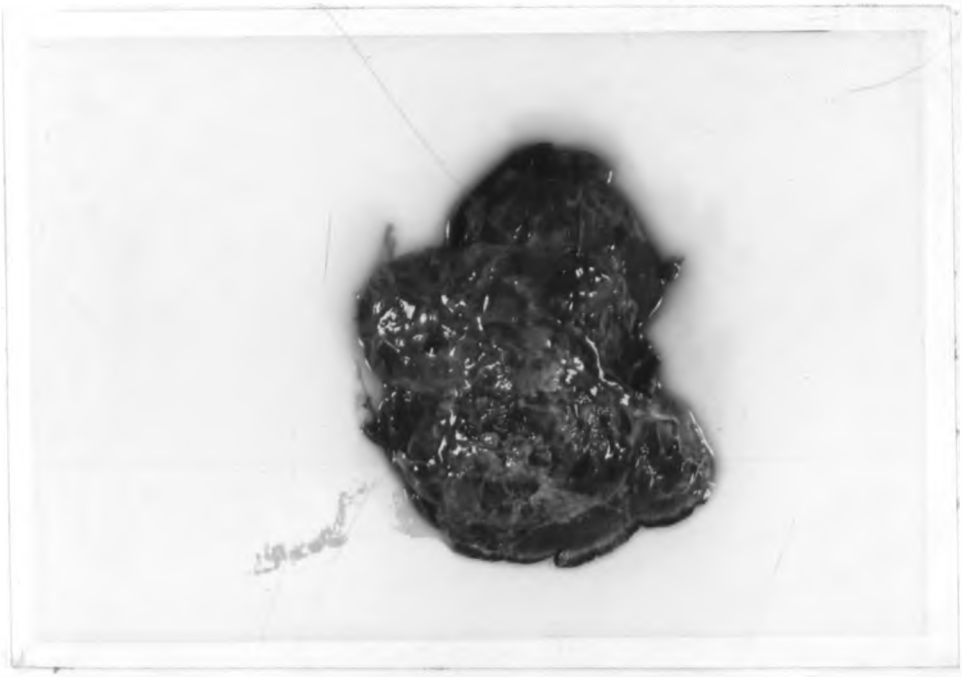

Fig. 12. A small liver of a male rat that had fed 1% Senecio powder for 15 months.

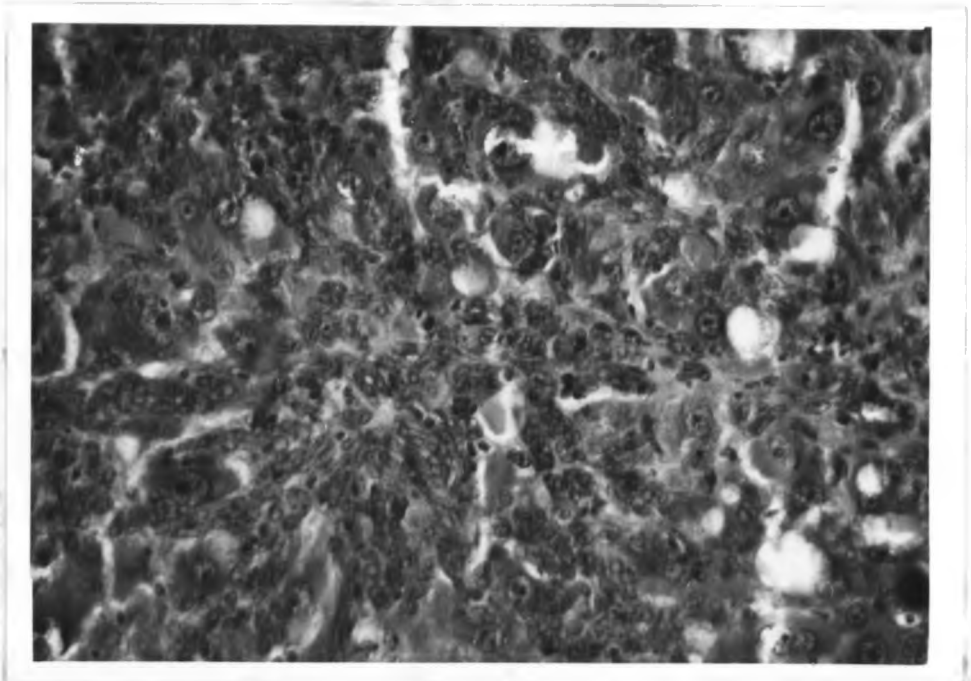

Fig. 13. Proliferation of endothelial cells in the liver of a rat fed 2% Senecio powder for 6 months. H&E Stain: X 391.

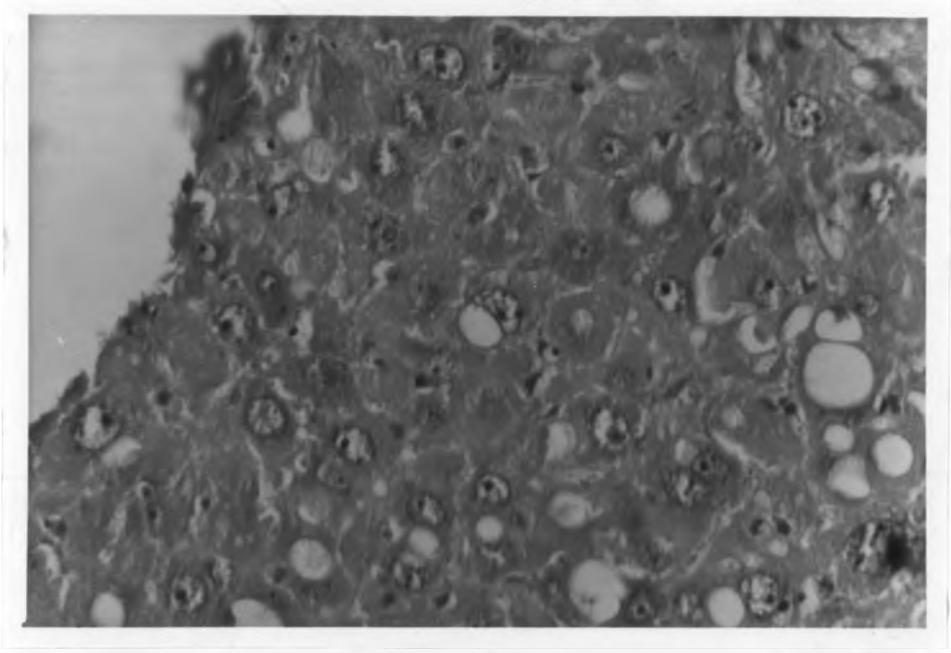

Fig. 14. Cytoplasmic vacuolation. Compression of a hepatic cell nucleus of a rat fed 3% Senecio powder for 5 months. H&E Stain: X 391.

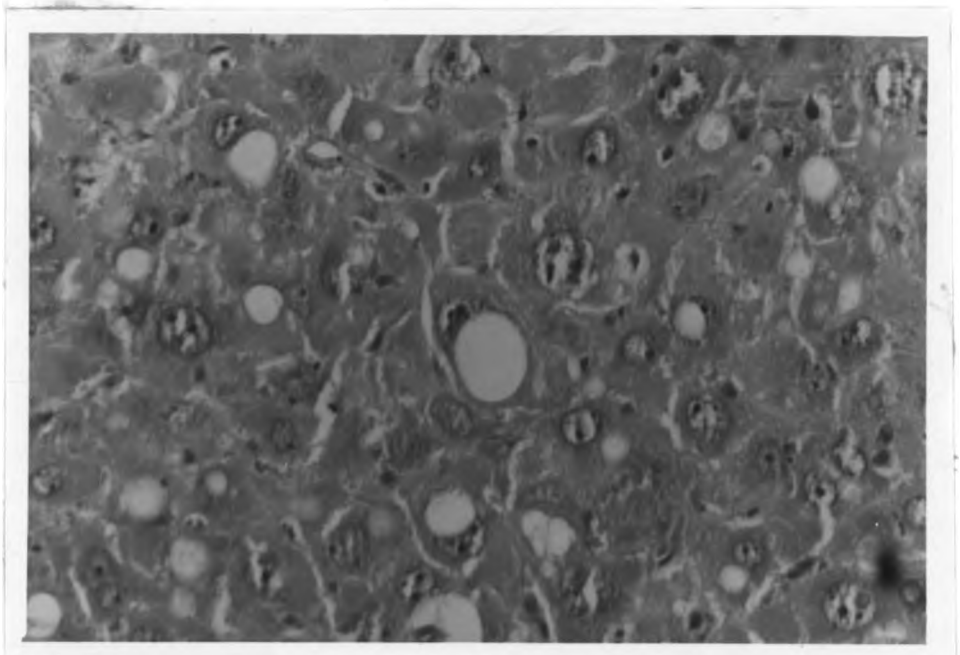

Fig. 15. Cytoplasmic vacuolation. Compresion of a hepatic cell nucleus of the same rat as Fig. 14; cresent appearance, H&E Stain: X 391.

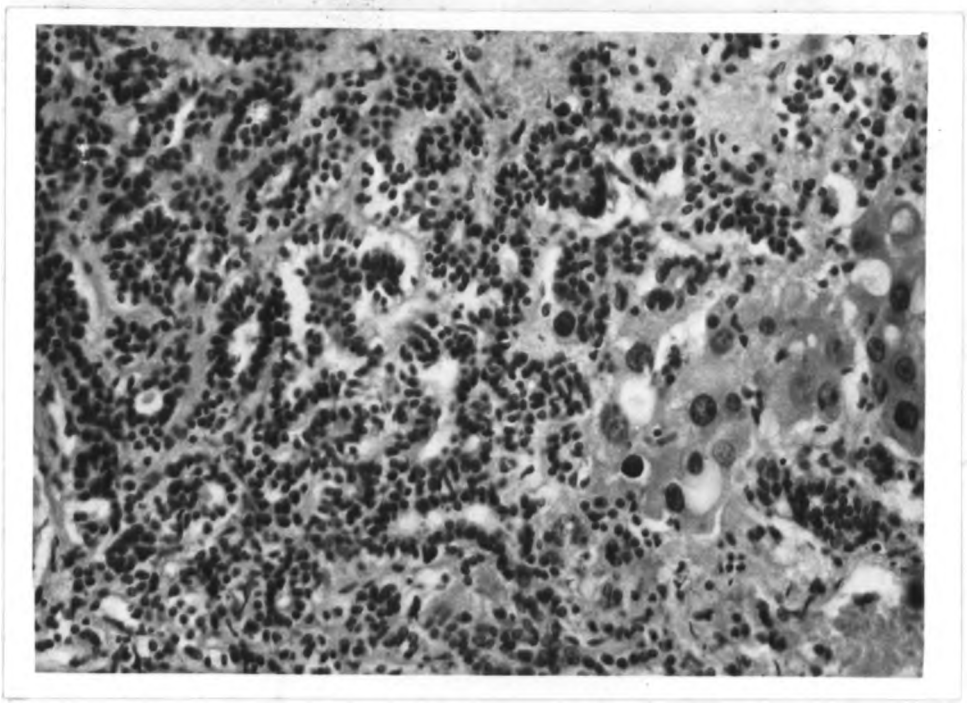

Fig. 16. Bile-duct hyperplasia. Liver secion from a rat fed 2% Senecio powder for 8 months. H&E Stain: X 156.

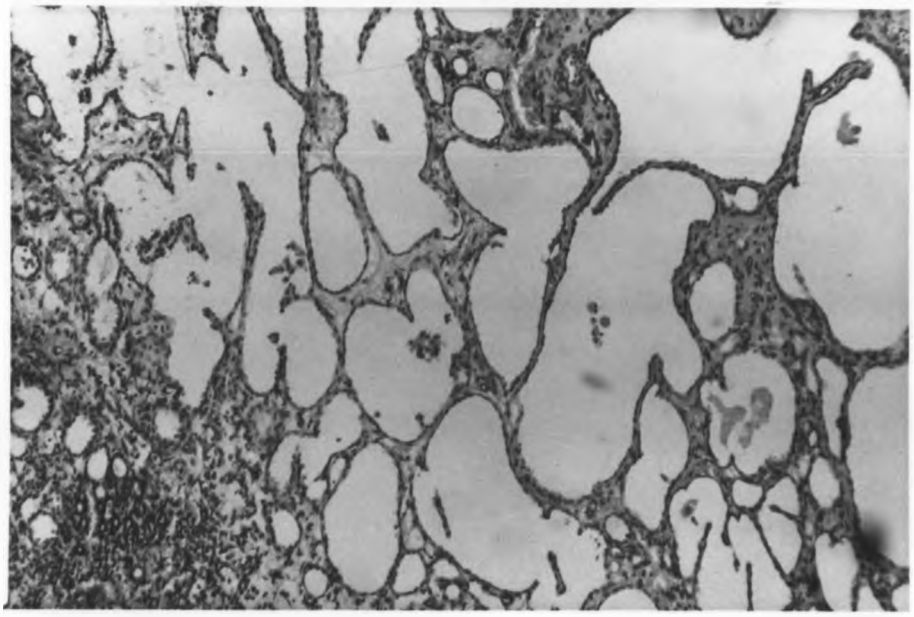

Fig. 17. Bile-duct cystadenorm. A section taken from a liver of a rat fed 1% Senecio powder for 13 months. H&E Stain: X 156.

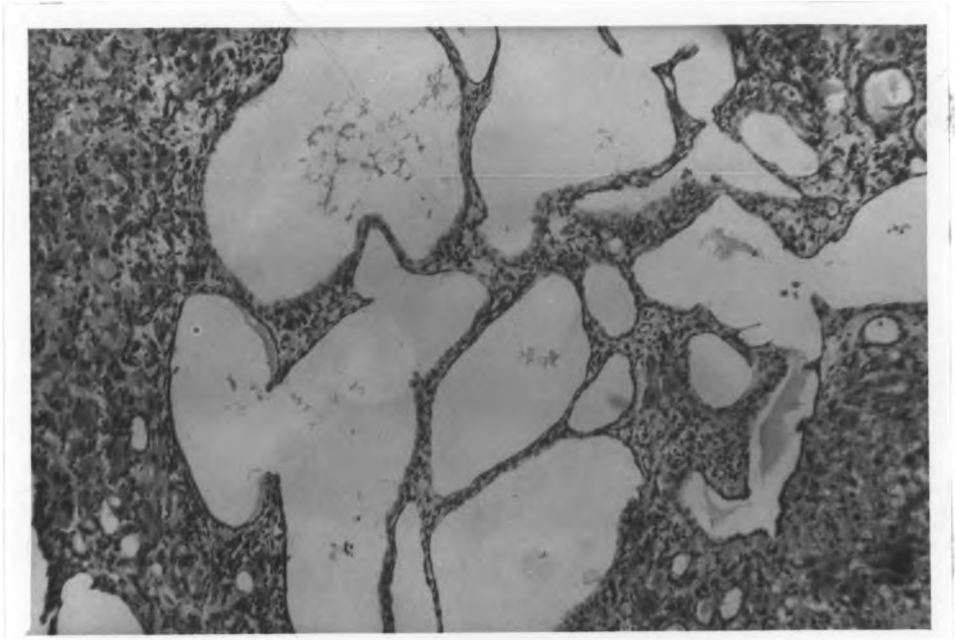

Fig. 18. Bile-duct cystadenoma. Section taken from the same liver as in Fig. 17. H&E Stain: X 156.

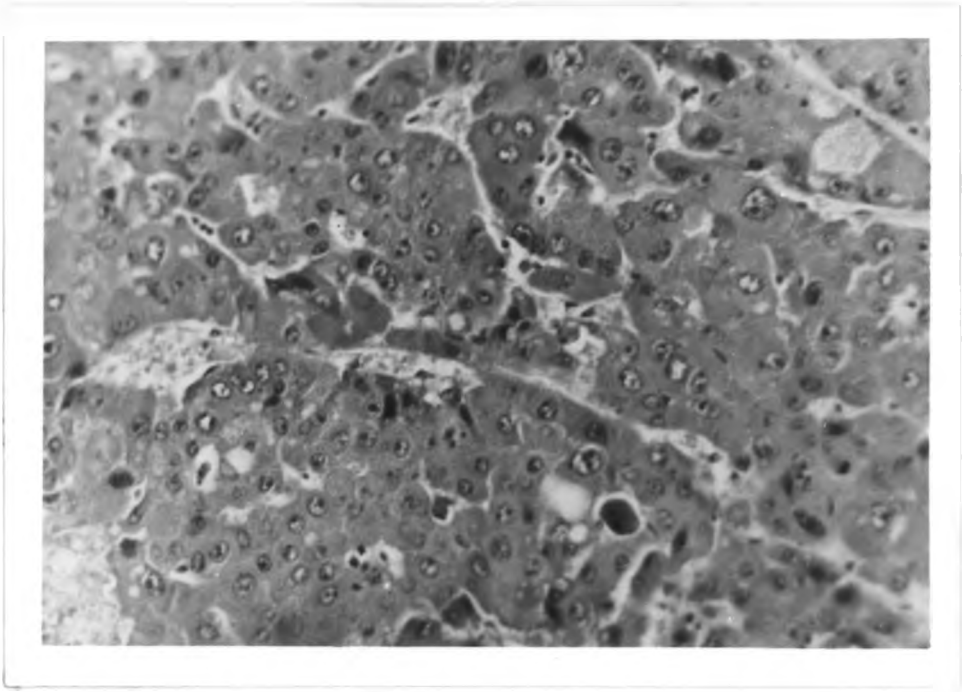

Fig. 19. Hepatoma. Section taken from liver in Fig. 11. H&E Stain: X 391.

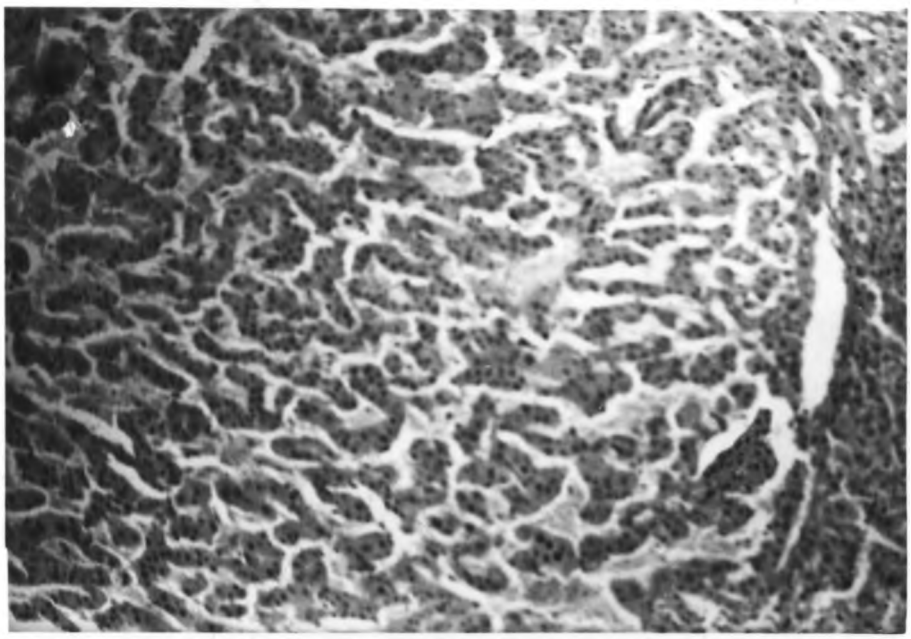

Fig. 20. A trabecular hepatoma. Section taken from liver in Fig. 12. H&E Stain: X 156.

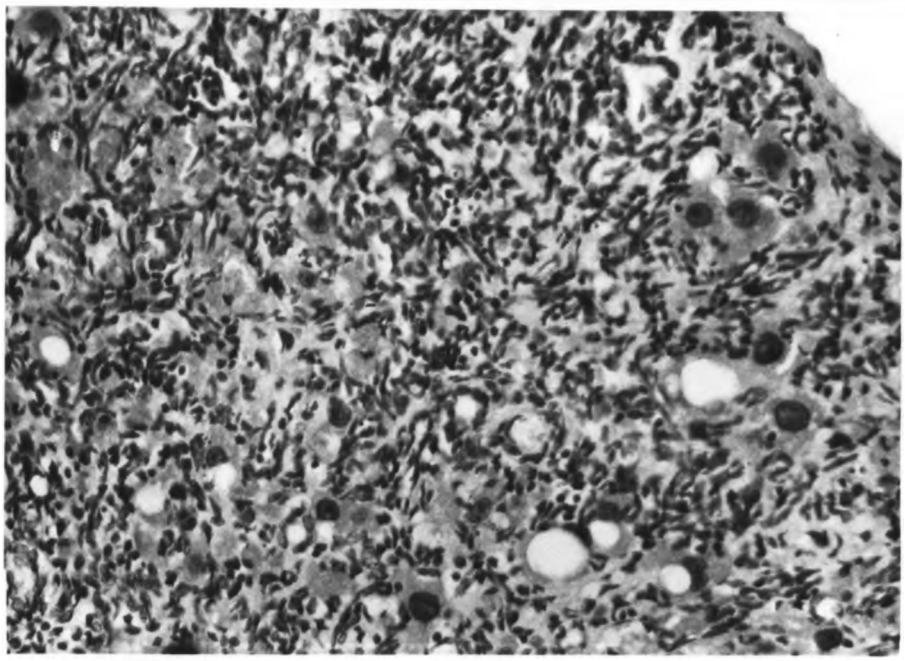

Fig. 21. Fibroma (F). Section taken from the liver in Fig. 12. H&E Stain: 391.

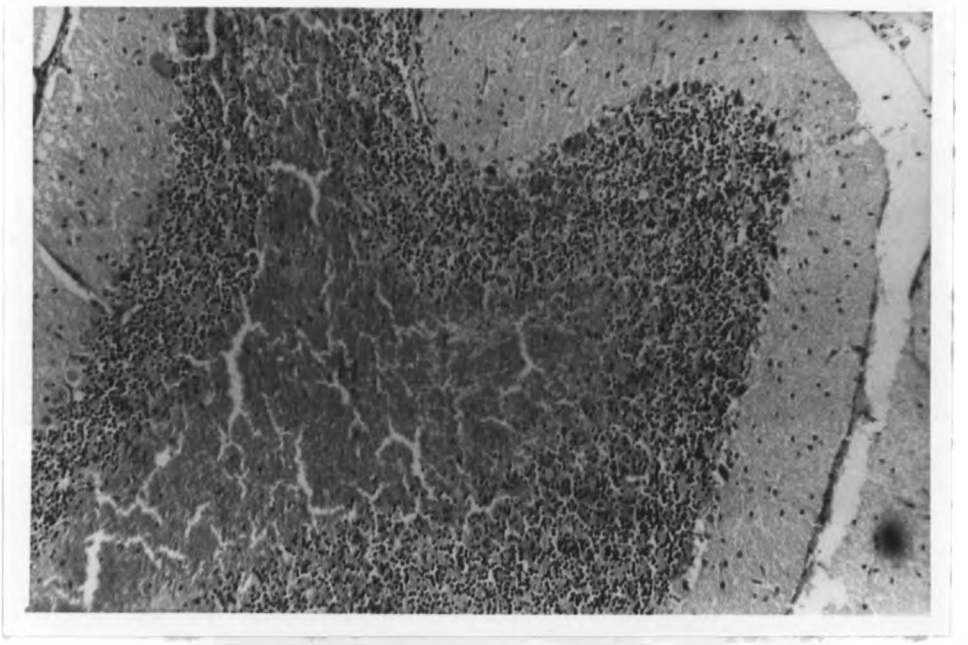

Fig. 22. Haemorrhage (H). Section from brain of a rat fed Senecio powder for 14 months. H&E Stain: X 391.

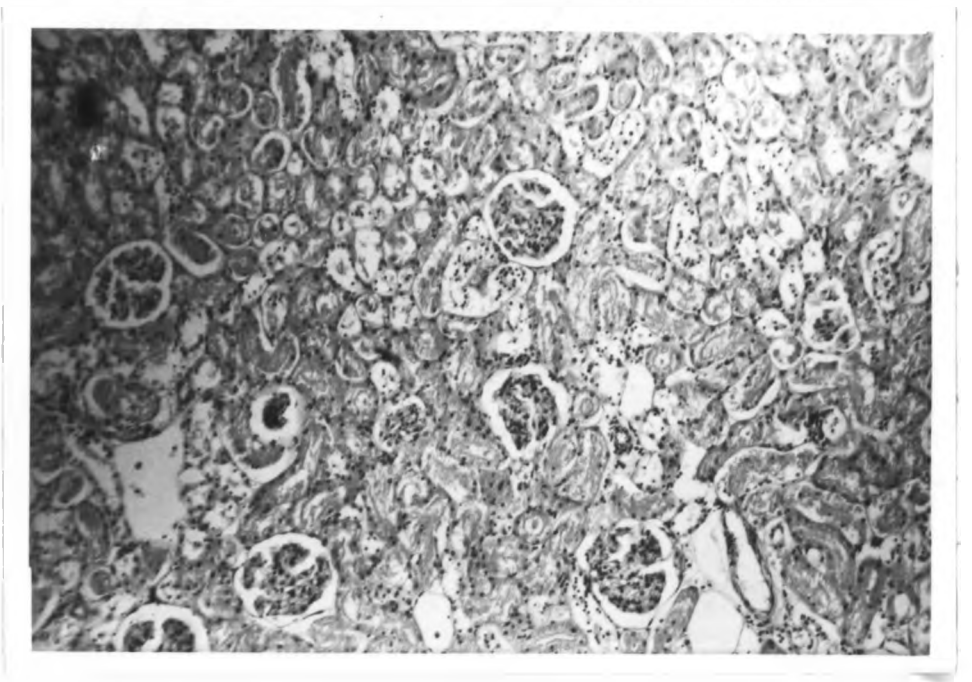

Fig. 23. Tubular necrosis of a kidney for a rat fed 1% Senecio powder for 10 months. H&E Stain: X 156.

Fig. 24. Exudate in the peritoneal (Ex.) cavity of a a bull calf drenched Senecio powder for 112 days.

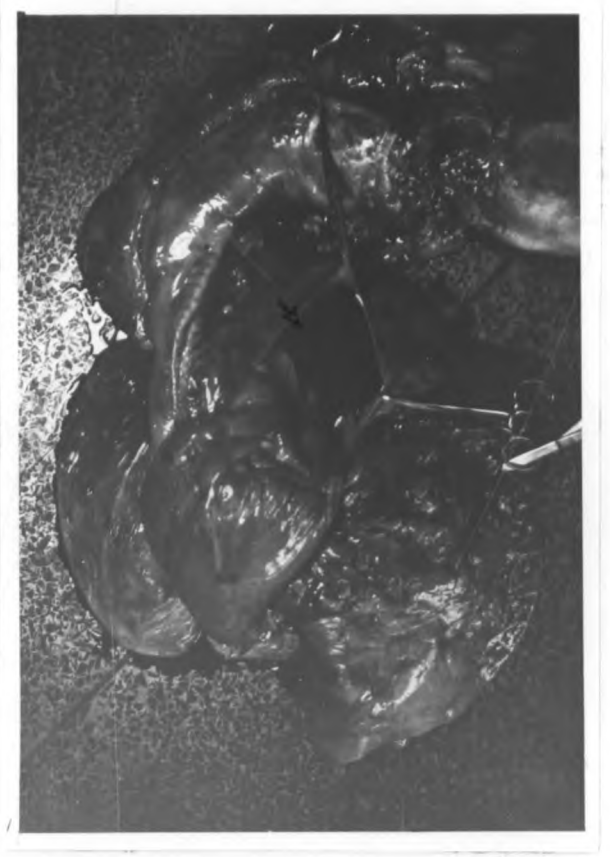

Fig. 25. Hydropericardium. The arrow shows exudate in the pericardium of bull calf No. 6688.

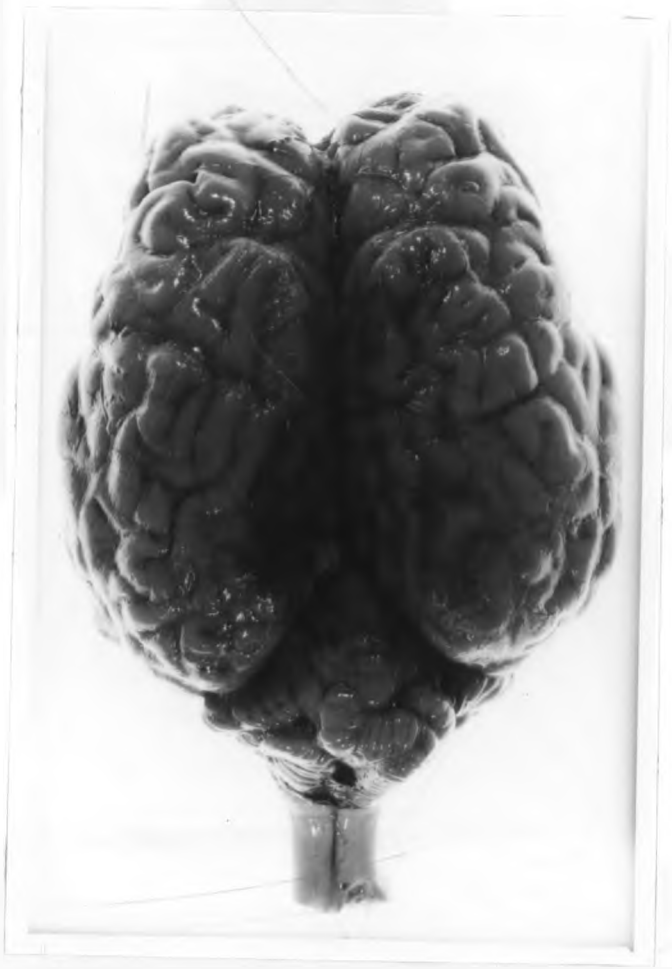

Fig. 26. Meningeal congestion in a bull calf drenched Senecio powder for 91 days.

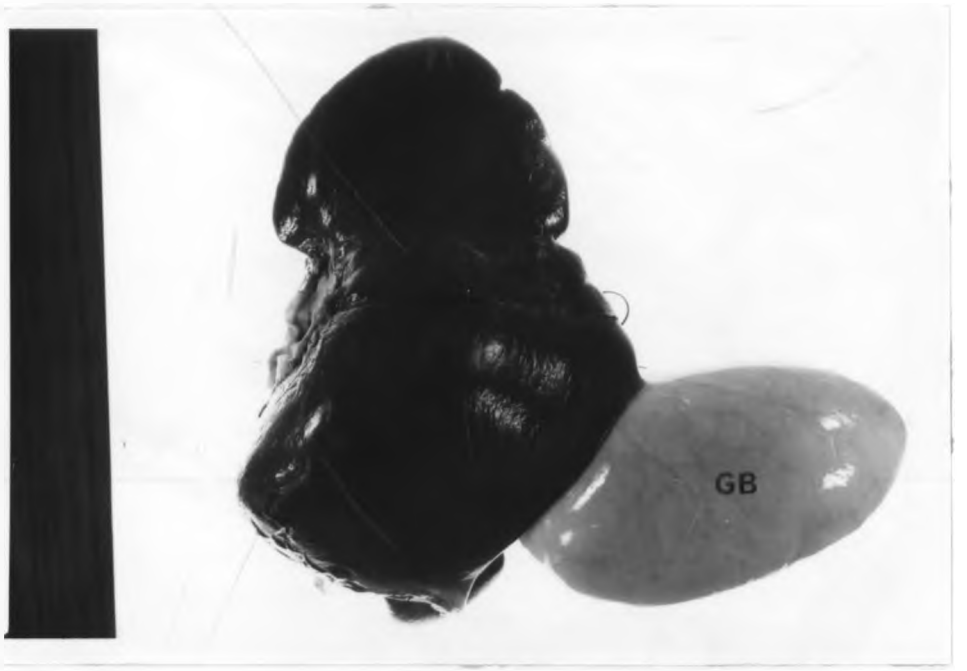

Fig. 27. An enlarged swollen liver with enlarged gall bladder (GB) of a bull calf referred to in fig. 26.

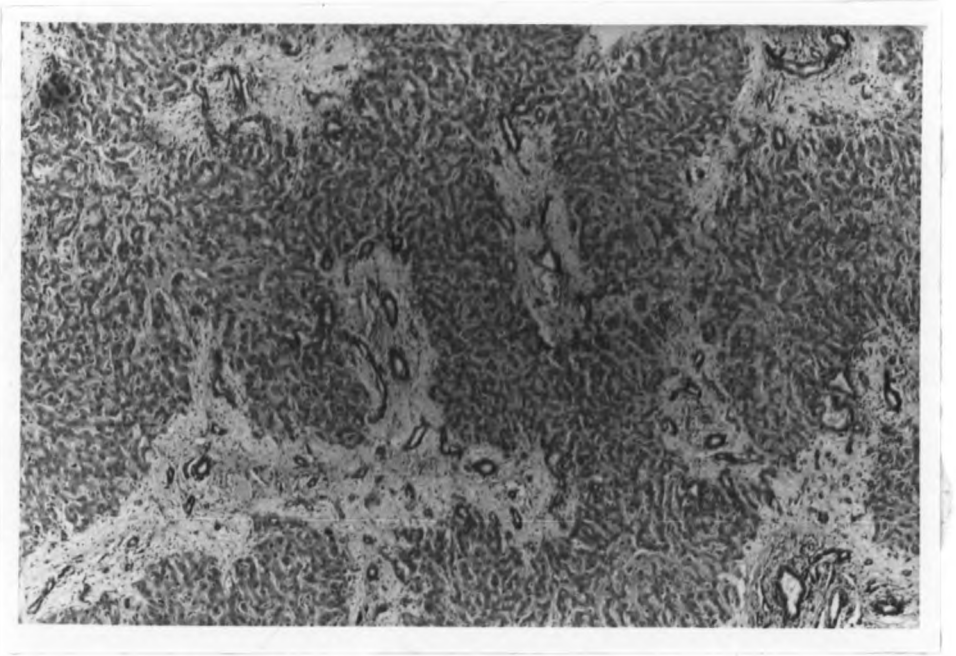

Fig. 28. Fibrosis. Liver section from a calf fed Senecio powder for 112 days. H&E Stain: X 156.

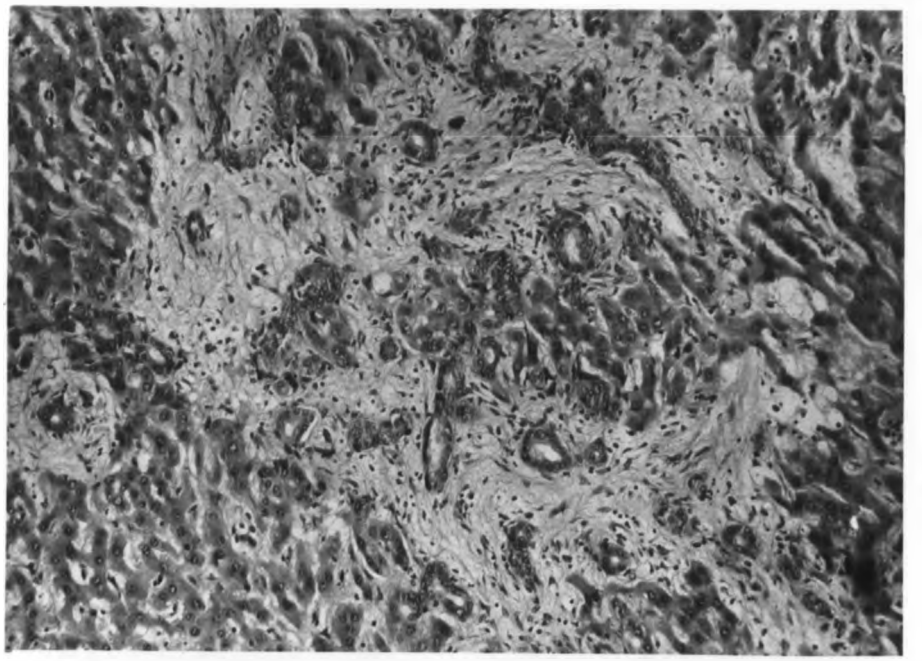

Fig. 29. Fibrosis. Section from the same liver as in Fig. 28. H&E Stain: X 391.

Fig. 30. Fibrosis. Thickened capsule and distorted lobular structure. Section from same liver as in Fig. 28. H&E Stain: X 63.

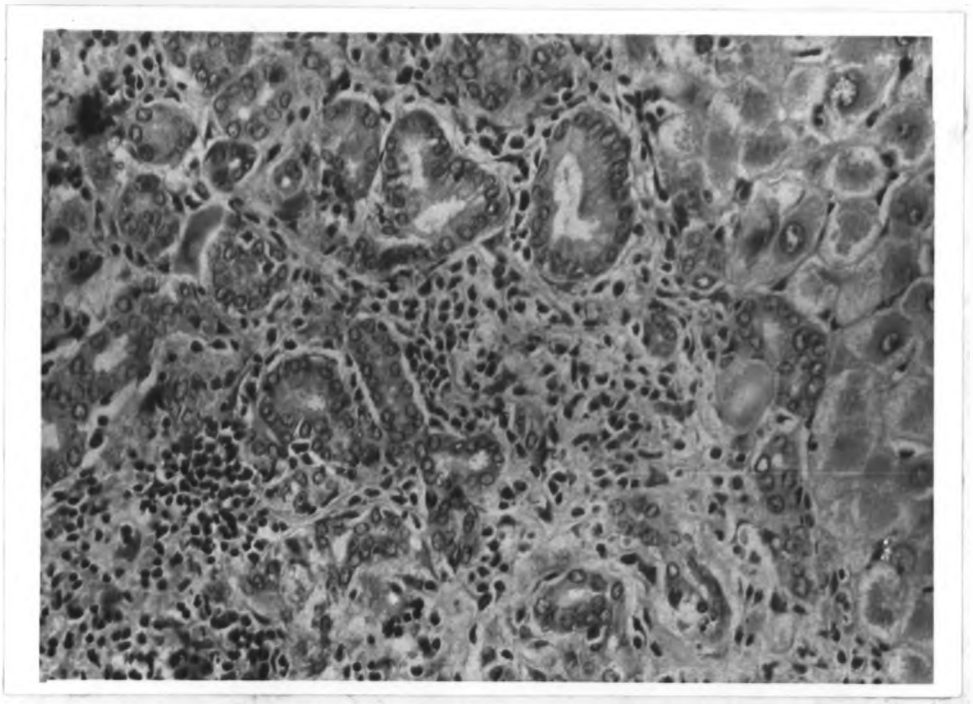

Fig. 31. Bile-ducts proliferation. Liver section of a bull calf drenched Senecio powder for 91 days. H&E Stain: X 391.

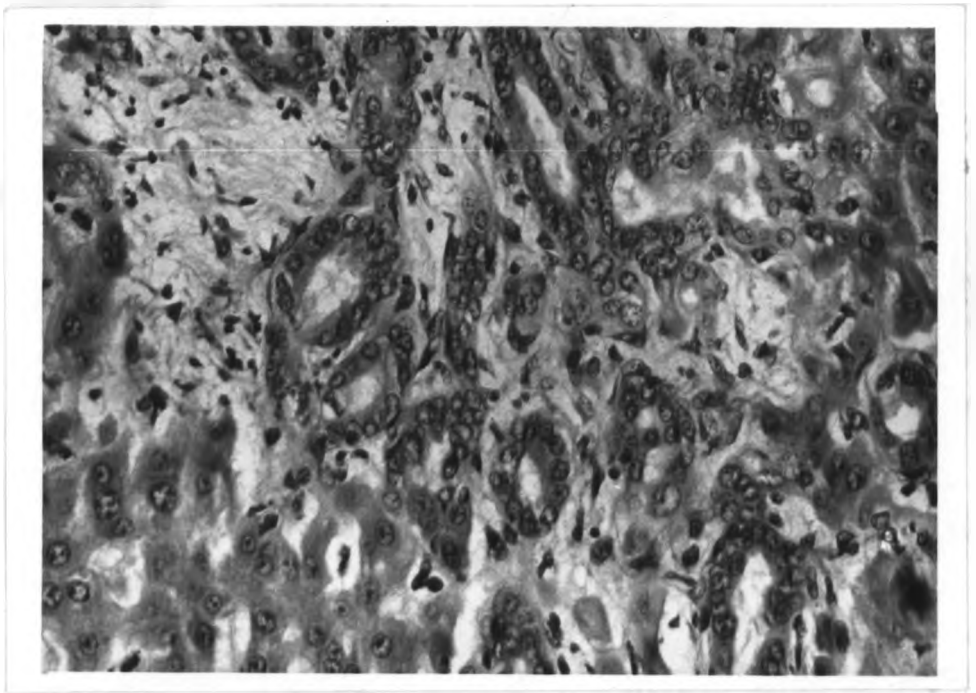

Fig. 32. Bile-ducts proliferation. Higher power of Fig. 31. H&E Stain: X 625

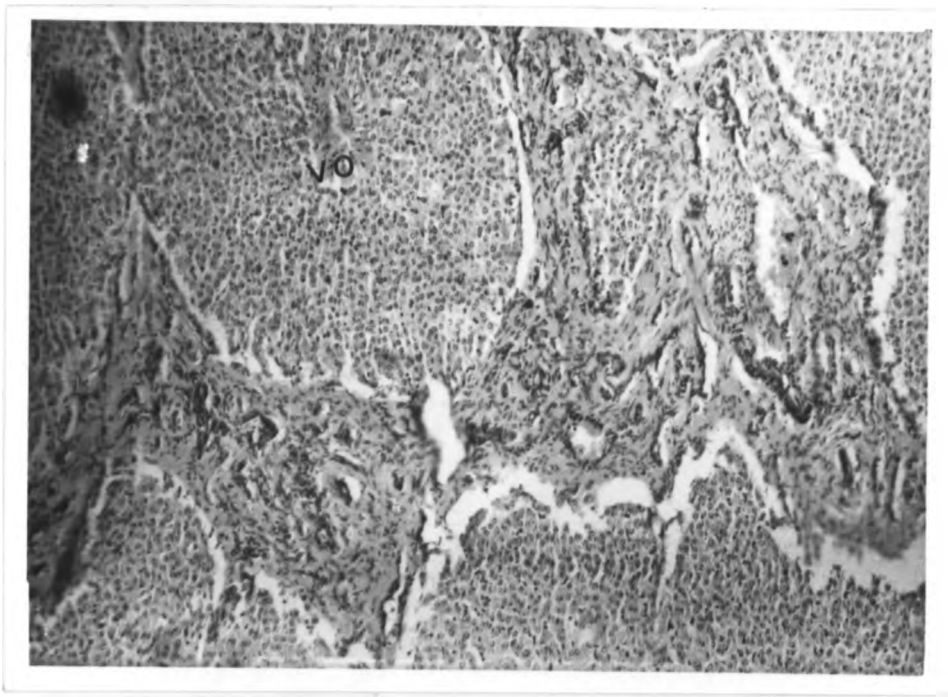

Fig. 33. Veno-occlusion (VO). Section from liver of a calf drenched Senecio powder for 112 days. H&E Stain:X 156.

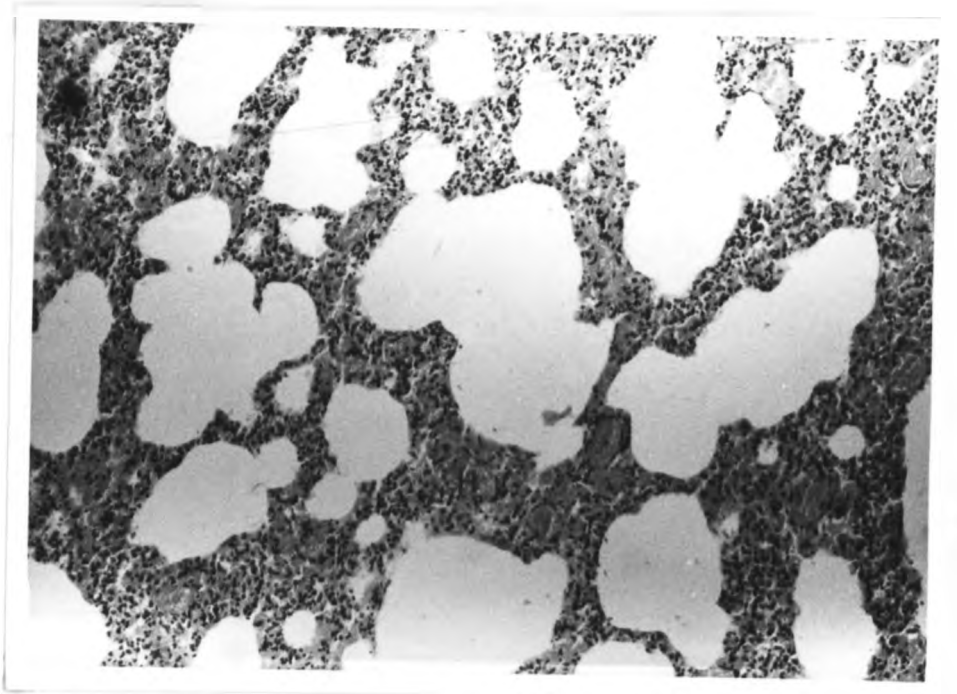

Fig. 34. Interstitial haemorrhage (H) and alveolar emphysema of a Bull Calf No. 6540. H&E Stain:X.156.

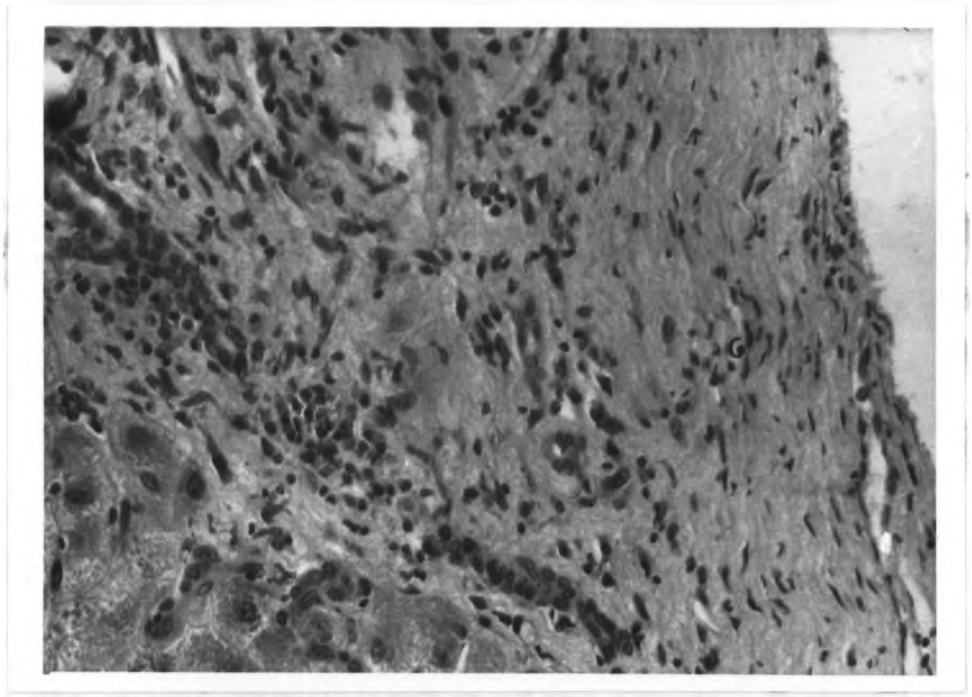

Fig. 35. Thickened capsule (C) and increased bile duct cells in the liver of a pig fed 25% Senecio powder for 60 days H&E Stain: X 625.

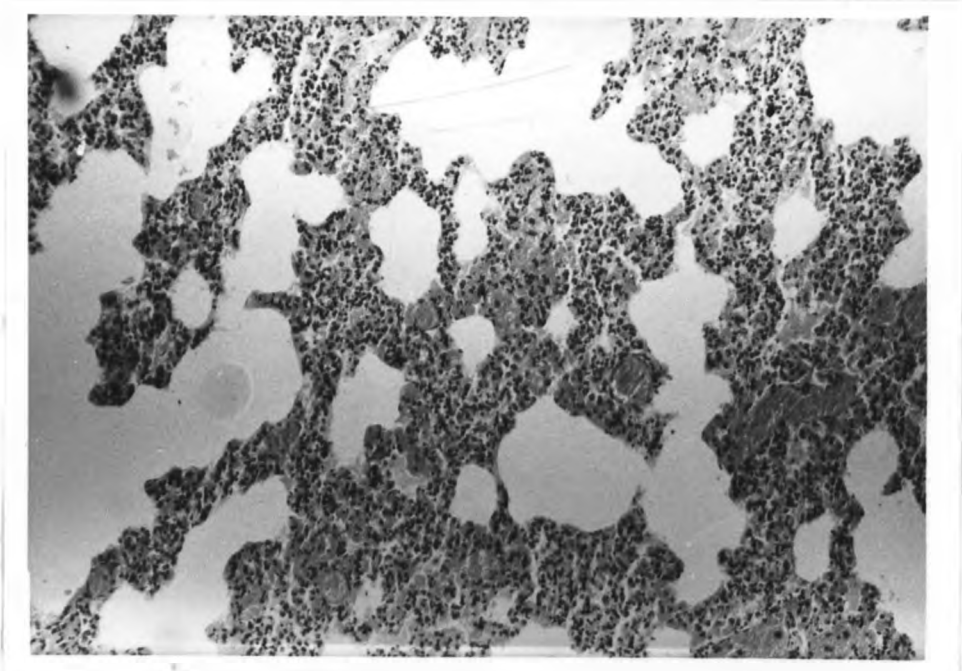

Fig. 36. Section from lung of a pig fed 25% Senecio powder for 60 days. Haemorrhage, slight oedema and emphysema. H&E Stain: X 156.

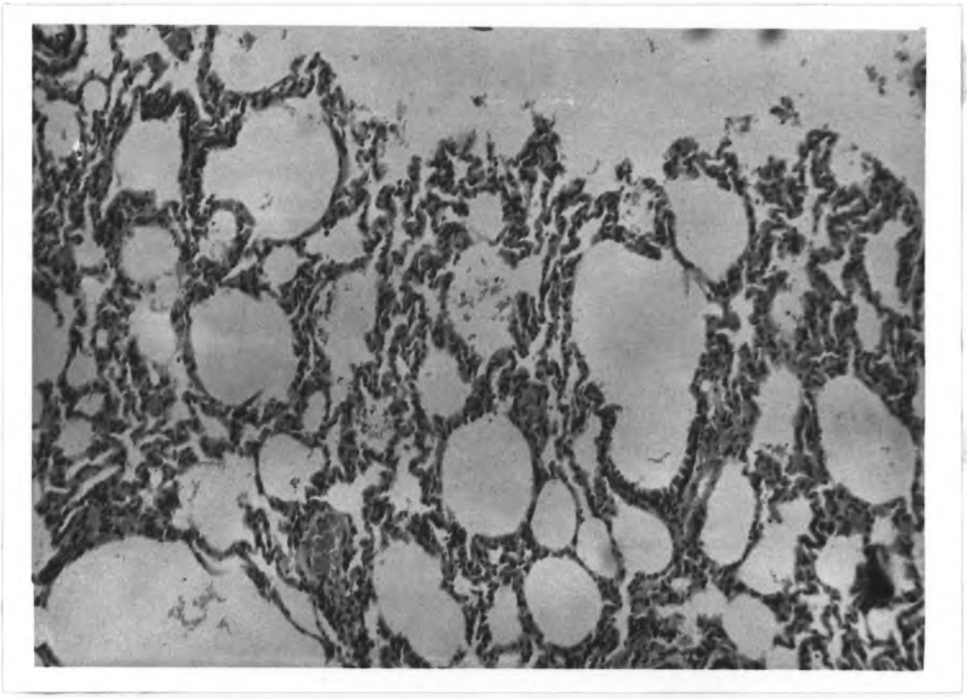

Fig. 37. Slight epthelialization of the alveoli. Section taken from same lung as in Fig. 36. H&E Stain: X 156.

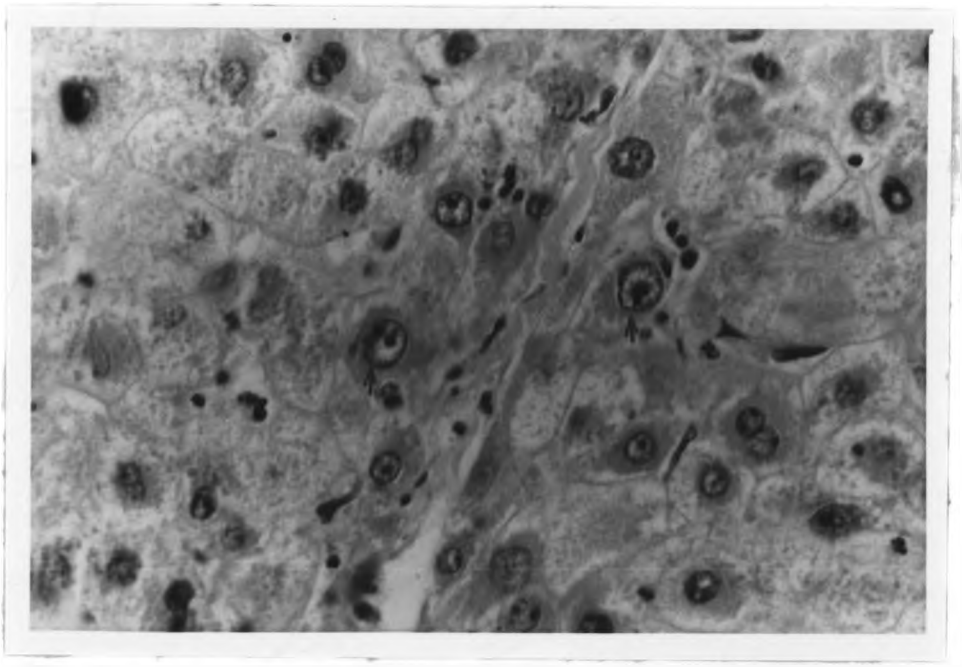

Fig. 38. Enlarged nuclei of the peripheral parenchymal cells, shown with an arrow. Section from a liver of pig fed 20% Senecio powder for 90 days. H&E Stain: X 391.

(b) ADENIA VOLKENSII TOXICOLOSIS IN ANIMALS

Experiment 1. Rat

MATERIALS AND METHODS

The plant material used in this experiment was collected from Mwala and Kilungu areas of Machakos District, Eastern Province of Kenya. The plants were uprooted, put in polyethylene bags and transported on the same day to the Faculty of Veterinary Medicine, Kabete. The tubers, stems, leaves and fruits were washed thoroughly, chopped off into very tiny chips and ground while still fresh into a thick powder in a standard No. 3 Willey Mill.

A group of sixty young locally bred Albino rats between 76 and 88 Gm were obtained from Kenyatta National Hospital. These rats were segregated by sex and divided into six groups of ten rats each. All the groups were housed in separate all metal cages. Four of these groups were fed with Adenia powder incorporated into basal diet to make the following percentages:

40%	-	first group
20%	-	second group
10%	-	third group
5%	-	fourth group

The fifth group was used as control and fed with commercially available chicken mash, the basal diet referred to above. The sixth group fed 50% Adenia powder incorporated into basal diet. However, Adenia powder here had been previously boiled for 1 hour and then dried. Water was available all

the time. The rats were wighed three times a week. Those in group six, however, were not weighed at all. All experimental and control animals were carefully observed daily and clinical signs recorded.

Routine necropsies were carried out on all animals that died naturally or were sacrificed when in extremis. Sections were taken from various organs, preserved in acetate-buffered 10% formalin. Routine sections were stained with haematoxylin and eosin.

RESULTS

TABLE 7 shows the number of rats alive each day of the experiment and TABLE 8 summarizes the difference in weight during the experimental period.

All rats, except those in group six that fed 50% boiled Adenia powder, and the controls were dead within the first two weeks of the experiment. At first these rats fed poorly and reduced much of their weights. After the first four days the loss of weight was not very sharp but progressive. The controls on the other hnd had a progressive increase of their weight. The first and the second group started dying on the third and fourth day respectively. They were all dead on the tenth day of experiment. Rats in group six although fed on highest percentage of Adenia powder (50%) did not die during the two week experimental period (TABLE 7).

Clinical Findings

Clinical symptoms of all rats that died were almost similar. The poisoning was of an acute type. At first the rats were noticed restless and had difficult breathing. They were noticed opening their mouths wide probably in an attempt to gasp the air. After one or two days of the onset of clinical signs diarrhoeic stool developed which persevered until death. Another significant clinical symptoms was reddish urine which was noticed from most of the rats that died. The cages had red spots scattered all over. From the second day of the onset of these symptoms general progressive weakness developed followed by staring coat, inability to move, coma and then death (Fig. 41). After the cessation of respiration, the heart beats continued for several minutes. Neither the controls nor the rats in group six developed the above symptom

Macroscopic Lesions

The macroscopic lesions were again very similar. In most cases, the animals were cachectic with diarrhoeic stool around the anal region and bloody spots on the tail. For the rats that died during the first five days of the experimental period, stomachs were almost empty.

In all cases no exudates were observed in either pleural or abdominal cavities. Haemorrhagic gastroenteritis with congestion of the mesenterium was observed in about 60% of the animals. In few cases only duodenum

and the intestines, particularly small, were inflammed. Lungs were in most cases bright-purple in colour. Liver, kidneys and spleen showed slight petechiation. Brain was in most cases hyperaemic. Urinary bladder of about 80% of the rats was distended with red content. On opening the bladder, congestion of the mucous membrane was noticed.

Microscopic Lesions

The microscopic lesions observed in all experimental rats were essentially similar inspite of varying percentages of Adenia powder used for each group and the variation in time of survival of the experimental rats.

Liver: The liver sections of most rats showed both venous and sinusoidal congestion, areas of haemorrhages and slight vacuolation of hepatic cells.

Kidneys; Nighty per cent of the experimental animals showed both congestion and haemorrhage of the renal cortex and medulla (Fig. 42). Slight vacuolation of the epithelial cells of proximal convoluted tubules and coagulative necrosis were observed in approximately 10 per cent of the experimental animals.

Lungs: The lesions observed in this organ were characterized by congestion of the organ and few areas of haemorrhage.

Spleen: Most animals did not show significant lesions in this organ

but 3 rats showed areas of haemorrhages, very marked in rat No. AD1 (Fig. 43).

Brain: Slight congestion and oedema of the meninges were observed in most 50 per cent of the experimental animals (Fig. 44).

Heart Muscle: The only lesion observed in this organ was sub-epicardial and intramuscular haemorrhage.

Gastro-intestinal Tract: Apart from haemorrhages and oedema observed in both mucous membrane and submucosa of the stomach and intestines (Fig. 45) there were no any other significant lesions seen.

The control rats killed at the end of the experimental period did not show any gross or microscopic lesions.

Experiment II. Rabbit

MATERIALS AND METHODS

Seven New Zealand White rabbits were obtained from Sadian Farm near Kabete and delivered to the Faculty of Veterinary Medicine, Kabete, Small Animal Unit. They were placed separately in all metal cages and fed with commercially prepared rabbit pellets and cabbages. Water was available all the time.

Five of these were assigned for experimental purposes while two were used as control. The experimental rabbits were drenched daily with varying amounts of cold aqueous extract of Adenia volkensii using stomach tube as follows:- No. 3605- 1.8ml./Kg. body weight, No.3677-3.3ml./Kg. body weight, No. 3315-5.4ml./Kg. body weight, No. 2566-7ml./Kg. body weight and No. 3370-13ml./Kg. body weight - (a single oral dose).

Numbers 3693 and 3563 served as controls. The rabbits were weighed at the beginning of experiment and at death.

Cold Aqueous Extract of Adenia

Tubers, stems and leaves of Adenia volkensii were washed chopped off and cut into very small pieces. These were crushed using pestle and mortar and extracted with cold distilled water at a concentration of 1 Gm. per 4 ml. water, leaving the soaked material for about 24 hours at 30°C. The mash was then strained, filtered through a Seitz Filter No. 2, centrifuged and stored at +4° until required.

RESULTS

The total amounts of Adenia extract used for each of these rabbits, variation in weight and the durations of drenching are recorded in TABLE 9.

Clinical Findings

The symptoms, which were almost similar in all rabbits inspite of variation in doses used started with loss of appetite, diffioult breathing, accelerated heart beat, profuse diarrhoea, nasal discharge, and protruding eye-balls.

Macroscopic Lesions

The lesions were again almost identical. On external examination the carcasses showed diarrhoeic stool around the anal region and two animals had nasal discharge. The animals had lost weight and were

cachectic. Three rabbits had little amount of exudate in both pleural and peritoneal cavities. Internal organs of all animals were hyperaemic and sometimes petechial haemorrhages and congestion were also observed (Fig. 46). Very hyperaemic were the mucous membranes of the trachea and bronchi. There were haemorrhages on the epicardium (Fig. 47), endocardium and the mesenterium. All rabbits had gastroenteritis and in two, haemorrhages in the stomach were also seen. In most cases the brain was hyperaemic.

Microscopic Lesions

Inspite of the variation in time of survival of the experimental rabbits the microscopic lesions were essentially similar.

Liver: Most animals showed severe sinusoidal and venous congestion, areas of haemorrhage, degenerative changes mainly cloudy and in one case destruction of the hepatic cells leading to necrosis.

Lungs: The lesions here were characterized by fibrinous exudates within the alveoli, congestion of the capillaries, haemorrhage (Fig. 48) and in one case thickening of the alveolar walls.

Kidneys: In almost all cases both the cortex and the medulla were congested. There were also areas of haemorrhages in both cortex and medulla in two cases.

Spleen: Sections of this organ from rabbit No. 3370 showed some haemorrhagic areas just beneath the splenic capsule.

Gastrointestinal Tract: Advanced oedema of the submucosa, congestion and sometimes haemorrhage were observed in all rabbits. Enteritis affecting particularly the large intestines was also a constant feature. In one case a severe gastroenteritis was observed.

Trachea: In two cases the mucous membrane of the trachea had haemorrhagic areas (Fig. 49). Submucosal congestion and oedema were observed as well.

Heart muscle: Slight haemorrhages were observed in the sub-epicardium in all five rabbits (Fig. 50).

Experiment III. Sheep

MATERIALS AND METHODS

A group of nine adult merino sheep weighing between 35 and 45 Kg. were obtained from the Department of Animal Production, Faculty of Veterinary Medicine - Kabete. The animals were divided into two groups of four sheep in one and five sheep in the other group. The group with four animals was used in the study of acute Adenia volkensii toxicity while that with five animals was used in the study of chronic A. volkensii toxicity. Both groups were housed in separate concrete floor houses where water, concentrates and lucerne were supplied ad libitum.

Experiment III (a): Acute Toxicity of Adenia volkensii in Sheep

Four sheep were drenched with single oral doses of varying amounts of Adenia volkensii powder suspended in cold tap water using bottle as

follows:- Sheep No. 301-40Gm., No. 300-20Gm., No. 275-10Gm. and No. 272-5Gm.

The preparation of Adenia volkensii powder was made as follows:- Tubers, stems, leaves and fruits of Adenia were thoroughly washed in cold water, chopped off and cut into tiny pieces. These were then sundried and ground into fine powder in a standard Willey Mill No. 3.

In this experiment no controls were kept. All the animals that died were examined at post-mortem and sections taken from various organs stained by haematoxylin and eosin for histological examination.

Experiment III (b): Chronic Toxicity of Adenia volkensii in Sheep

Sheep numbers 279, 274 and 302 were injected daily with an aqeous extract of Adenia volkensii. Sheep numbers 298 and 271 were kept as controls. The aqeous extract was prepared as described above in the Rabbit Experiment. These experimental sheep were given daily intravenous injections with extract as follows:-

Sheep No.	Amount injected in ml. per Kg. Body Weight	Days of injection
279	0.01	22
274	0.02	17
302	0.03	18
298	-	-
271	-	-

Daily temperatures, pulse rate, and respiration rate were taken from each of the experimental sheep as well as controls. Blood samples for serum enzyme determination were also taken daily.

Serum samples were examined for Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxalacetic Transaminase (SGOT) and Alkaline Phosphatase (A.P.). SGPT and SGOT were determined by the method of Reitman and Frankel (1957) and expressed in Sigma Frankel Units per ml. Alkaline phosphatase was determined according to the method of Kind and King (1954) and readings expressed in King-Armstrong units per 100 ml. Animals were examined daily and all that died as well as the sacrificed controls were examined by standard necropsy procedure. The tissues were collected in buffered 10% formalin. Routine sections were stained with haematoxylin and eosin.

RESULES

Experiment III (a)

TABLE 10, summarizes the duration after drenching in which the sheep were alive. Sheep numbers 301, 300 and 275 died within twenty four hours of drenching. Before death they seemed depressed, had poor appetite and accelerated pulse rate (140-160 per minute). No diarrhoea had time to develop and only in one sheep that mucoid nasal discharge was noticed. The fourth sheep died in the fourth day after drenching.

It had developed a watery diarrhoea, mucoid nasal discharge, accelerated weak pulse rate and accelerated respiration rate (70-80 per minute).

Macroscopic Lesion

Three of the experimental sheep showed epicardial and endocardial haemorrhages more pronounced on the left side of the heart. The sheep No. 272 had haemorrhages on the intestinal mucosa especially large intestine and caecum. Lungs looked congested grossly. This sheep also showed hyperaemia of the internal organs, meninges and the mucous membrane of the trachea.

Microscopic Lesions

Liver: Sections from most livers showed congestion as well as some areas of haemorrhages.

Kidneys: Characteristic lesions were congestion, vacuolation of renal epithelial cells and some areas of haemorrhages.

Lungs: There were scattered areas of haemorrhages, congestion and alveolar exudate in most sections observed.

Brain: Congestion of the meninges and brain capillaries.

Heart muscle: Some degenerative changes especially hyaline were noticed. The fourth sheep showed inflammation of the mucous membranes of abomasum and intestines.

Experiment III (b)

Sheep No. 274 died on the 17th day of treatment. On the tenth day, the animal had already shown poor appetite, slight bloat, frequent urination, recumbency and depression. The condition worsened during the following days and a day before death mucoid nasal discharge and diarrhoea developed. On the 18th day of treatment, sheep No. 302 died. It showed essentially similar clinical symptoms as described in sheep No. 274. The last sheep (No. 279) died on the 22nd day of the experiment. From the 15th day appetite was poor it became emanciated and on the 20th day mucoid diarrhoea developed which lasted until death. Other clinical signs were almost identical to those shown by sheep No. 274. The controls which were sacrificed on the 23rd day of the experiment did not show any of the above clinical symptoms.

The temperatures, pulse rate, respiration rate, and serum enzyme values are indicated in TABLES 11, 12, 13, 14, 15 and 16 respectively. As indicated, there were no significant changes in temperatures, pulse rate and respiration rate. There was also no significant rise in serum enzymes in experimental animals as compared to the controls.

Macroscopic Lesions

The macroscopic lesions of all experimental sheep that died were again essentially similar. In all cases hyperaemia of the internal organs was noted. The mucous membrane of the trachea and the brain

were also hyperaemic.

Characteristic petechial haemorrhages were observed on the epi-and endocardium, lungs, diaphragm, spleen and the peritoneum. The mucosa of the stomach and that of the intestines were inflammed in all three animals.

Microscopic Lesions

There was no much variation of the microscopic lesions seen in different organs inspite of varied dosages used for each experimental animal and the duration of treatment.

Liver: There was a moderate but well-defined distension of the sinusoids and of the portal, central and sublobular veins as well as of the branches of the hepatic artery, as a result of congestion. Few sections showed haemorrhage and slight vacuolation of the hepatic cells.

Kidneys: Sections from most kidneys were congested. There were haemorrhages particularly marked in the medulla. Cortex showed necrosis of the convoluted tubules characterized by marked reduction of the nuclei as well as varying degree of cloudy swelling of the tubular epithelial cells and hyaline casts deposition in the tubules (Figs. 51 and 52).

Lungs: Marked congestion and sometimes alveolar oedema was characteristic of all sections observed. Two sections showed some inflammatory cells with greatly increased mononuclear cells.

Heart Muscle: Sub-epicardial haemorrhage was noted in all sections. In one case there was also dystrophy of muscular fibres of the heart.

Spleen: There were no significant lesions seen.

Gastrointestinal Tract: This showed catarrhal enteritis affecting particularly the jejunum (Fig. 53).

Brain: There was slight congestion and oedema of the meninges (Fig. 54).

The control animals did not show any lesions, grossly or otherwise.

DISCUSSION FOR PART III (b)

In certain countries the poisoning of stock by cyanogenetic plants reaches proportions of considerable economic importance. In South Africa a wide range of these plants occurs, causing mortality especially in sheep. This stimulated a great deal of investigational work by the Onderstepoort workers who have listed the plants dengerous to stock (Steyn 1934) and have determined many factors influencing the toxicity of these plants to stock. American work has resulted largely from the widespread use of Sorgum and Sudan grass in stock feeding, these two species containing Cyanogenetic glucosides which may under certain conditions reach toxic proportions (Moran 1954). In Australia and New Zealand the problem of cyanide poisoning arises mainly from the presence of Cyanogenetic glucosides in Sudan grass and White Clover (Coop et al. 1950). The present experimental study in rats, rabbits and sheep, using Adenia volkensii, has shown that this plant which is widely distributed in East African countries is dangerously toxic to these species of animals. Hydrocyanic acid has also been isolated from

this plant (See Part IV) and is considered to be one of the most important factors associated with its toxicity. It therefore may well be counted among the cyanogenetic plants in East Africa.

A great many plants are capable of producing HCN sometime during their growth. Quisumbine, quoted by Moran (1954), lists over 300 separate species distributed into 74 different families of seed-bearing plants which he says are known to contain HCN. Hydrocyanic acid or prussic acid is one of the most toxic and rapidly acting of the common poisons. It is poisonous to any type of animal because it affects respiration, paralyzes nerves and interferes with normal physiological functions (Pammel quoted by Moran 1954).

Animals poisoned by HCN show signs of suffocation caused by tissue hypoxia since hydrocyanic acid inhibits the enzyme system necessary for transport of oxygen from blood to the tissue. The particular signs observed will depend on the size of the dose and the time over which it is absorbed. Moran (1954) states that at post-mortem there is no characteristic gross or microscopic lesion which would definately point to HCN-poisoning. However, he states that dark muscle, tissue congestion or haemorrhage of the lungs, petechiation of the tracheal mucosa and a mucoid, frothy or bloody discharge from the mouth and nostrils would be suggestive to HCN poisoning. The clinical signs and post-mortem lesions encountered in the present study were all suggestive of an acute HCN

poisoning, as a result of which analytical work was carried out and the concentration of HCN present in Adenia volkensii estimated (See Part IV).

The minimum lethal dose of free hydrocyanic acid for most species when given per os is of the order of 2.0-2.3 mg. HCN per Kg. body weight (Garner 1967). It is not, however, possible to state with any certainity the toxic dose of cyanide in the form of cyanogenetic glycoside, as this varies according to the conditions obtaining in the plant (highest concentrations are seen in young, actively growing plants), and in the animal at the time of eating the plant (Van der Walt 1944). The same author states that poisoning in ruminants depend upon the quantity of the plant ingested, the previous diet of the animal, the pH of the stomach contents, the percentage of total hydrocyanic acid present in the free state in the plant, the concentration of cyanide liberating enzyme present in the plant and the total hydrocyanic acid content in the plant. The importance of these factors has been enlarged up by Coop et al. (1950). In addition to this it would also appear that although Adenia volkensii was shown to contain very large cyanide concentration, particularly in the tuberous root stock (See Part IV) the process of sun drying, grinding and extraction reduced the concentration of cyanide in the plant material. This would help to explain why in the present study the relatively high doses and prolonged period of time were necessary before the experimental animals died.

Whether there is any chronic effect of HCN on livestock is questionable. Moran (1954) states that when an animal recovers from a sublethal dose of HCN, it is usually considered to have no effect on a later dose. As such, cyanogenetic plants, probably produce no real chronic stock poisoning similar to that of the Senecio spp. or other plants that produce a cumulative effect. Though some authors - De Gier, Heffter etc., quoted by Van der Watt (1944), believe that chronic poisoning is possible, it is generally recognized that provided HCN or Cyanogenetic plants are ingested at a moderate rate throughout the course of the day, animals can tolerate amounts well in excess of the MLD for a single dose. Van der Walt (1944) failed to produce chronic poisoning in sheep even after administering 3.2mg. HCN/Kg. daily for 2 years. Eckell, quoted by Moran (1954), gave daily doses to a horse starting with small sublethal doses and gradually increasing the dosage in 20 days, to what was considered a minimum lethal dose without producing sysmptoms. Worden (1940) showed that in the rabbit repeated dosing does not produce a cumulative effect and that the animal is capable of eliminating $\frac{1}{2}$ MLD at $2\frac{1}{2}$ hours.

Although there may be some evidence that chronic cyanide poisoning may be induced experimentally in monkeys and in sheep (Hurst, quoted by Garner 1967), there is no reason to believe that it ever occurs under field conditions. According to Hindmarsh (1941) prussic acid is not

a poison to which we would expect tolerance to develope. It is quickly absorbed and if the dose is not lethal, it is rapidly destroyed in the body or excreted in the urine as sulphocyanide. In the present study, three sheep were given daily intravenous injections with varying amounts of Adenia extract in an attempt to produce chronic poisoning. In spite of the fact that the animals died between the 17th and 21st day of the experiment, neither the clinical symptoms nor the post-mortem lesions were suggestive of any possibilities of chronic poisoning. As Van der Walt (1944) puts it, injury by HCN to nerve tissue, blood corpuscles, or other tissues of cells which lower resistance of animals to more poison might be interpreted as a chronic effect.

Boiling of the tuber of Adenia volkensii reduces its toxicity considerably. This fact has been confirmed by feeding experiments on laboratory animals (See Rat Experiment). Preparation of most herbal medicines used by Africans involve boiling, sometimes for several hours. It is probable that for this reason, the plant has been used without fatalities

TABLE 7

Daily Mortality of Rats Fed on Varying Percentages of Adenia Powder.

Group	Days of the experiment and number of rats alive													
	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21th	22nd
1	10	10	10	8	8	7	5	3	1	-	-	-	-	-
2	10	10	10	10	8	8	6	3	2	1	-	-	-	-
3	10	10	10	10	10	10	8	7	6	2	1	-	-	-
4	10	10	10	10	10	8	8	6	6	4	3	3	2	1
5	10	10	10	10	10	10	10	10	10	10	10	10	10	10
6	10	10	10	10	10	10	10	10	10	10	10	10	10	10

TABLE 8

Weight Changes in Rats Fed on Varying Percentages of Adenia Powder

Group	Experimental dates and weight in Gm.						
	9.3.73	12.3.73	14.3.73	16.3.73	19.3.73	21.3.73	22.3.73
1	817	792	607	167	-	-	-
2	789	768	628	243	-	-	-
3	792	777	742	531	61	-	-
4	812	807	773	505	159	81	43
5	768	788	801	817	829	831	832

TABLE 9

Weight Changes and Total Amounts of Adenia Extract Given to Each Experimental Rabbit

Rabbit number	Initial weight in gm.	Amount drenched in ml./kg. body weight	First day	Last day	Total amount in ml.	Weight at death in gm.	Weight gain/loss in gm.
3605	2715	1.8	23.3.73	27.3.73	25	2671	-44
3677	3010	3.3	23.3.73	25.3.73	30	2961	49
3315	2801	5.4	23.3.73	25.3.73	30	2768	33
3566	2800	7	23.3.73	24.3.73	40	2764	36
3370	3012	13*	24.3.73	25.3.73	40	2958	54
3693	2719	-	23.3.73	27.3.73	-	2975	256
3563	2639	-	23.3.73	27.3.73	-	2760	121

* Single oral dose.

TABLE 10

Amount of Adenia Powder Given to Each Sheep and Duration that Each Sheep Lived After Drenching

Sheep number	Adenia powder in gm.	Duration in hours
301	40	12
300	20	15
275	10	23
272	5	96

TABLE 11

Daily Temperature Readings in Sheep After Injection

With Adenia Extract

Day of Injection	Temperature of the animal in °C				
	279	274	302	298*	271*
1	37.5	38.3	39	38.5	38.9
2	37.8	37.9	38.8	38.2	39.4
3	39	37.6	38.3	37.1	39.4
4	38.3	38.3	37.9	37	39.4
5	38.1	38.8	37.8	38.1	38.9
6	37.8	38.9	38.5	39	39
7	38.9	39.4	38.3	39.4	38.5
8	38	38.9	38.3	39.1	38.3
9	39	38.3	38.3	39.4	38.3
10	38.1	38.9	38.7	39	38.9
11	39	38.8	37.9	38.3	38.3
12	39	37.8	38.2	38.5	38.1
13	38.9	38.6	38.3	39	38.9
14	39	38.9	38.3	39.4	38.9
15	39.3	38.3	37.9	38.8	39.4
16	38.7	37.8	38	38	39
17	38.7	-	38.3	37.9	39.1
18	38.2	-	-	38.3	38.5
19	38	-	-	38.9	39
20	37.9	-	-	38.5	38.6
21	37.2	-	-	38	38.1
22	-	-	-	38.3	38.7
23	-	-	-	38.3	38.7

* Controls

TABLE 12

Daily Pulse Rate in Sheep After Injection with Adenia Extract

Day of injection	Pulse rate per minute				
	279	274	302	298*	271*
1	80	80	76	85	70
2	90	80	90	100	70
3	80	84	86	90	82
4	80	80	88	98	76
5	78	80	88	70	70
6	90	96	90	80	80
7	86	90	86	80	82
8	90	70	84	86	80
9	90	80	86	70	80
10	90	84	80	90	80
11	88	86	90	90	94
12	80	90	88	100	100
13	70	90	84	84	80
14	80	86	82	90	84
15	100	94	90	78	86
16	90	96	96	80	86
17	90	-	92	84	82
18	84	-	-	86	90
19	82	-	-	88	94
20	96	-	-	88	84
21	100	-	-	90	82
22	-	-	-	94	86
23	-	-	-	88	84

* Controls

TABLE 13

Daily Respiration Rate in Sheep After Injection with Adenia Extract

Day of injection	Respiration rate per minute				
	279	274	302	298*	271*
1	24	20	24	24	24
2	24	20	24	24	24
3	24	24	24	24	24
4	24	20	24	24	20
5	26	24	23	20	24
6	20	24	24	24	24
7	24	25	22	24	23
8	24	24	24	24	24
9	22	24	24	26	24
10	20	20	25	24	22
11	24	22	23	26	24
12	22	23	23	24	24
13	25	26	24	24	25
14	24	26	23	23	22
15	24	25	24	24	24
16	22	26	24	24	24
17	20	-	25	26	24
18	24	-	-	24	23
19	24	-	-	22	25
20	25	-	-	20	24
21	25	-	-	22	22
22	-	-	-	24	24
23	-	-	-	24	24

* Controls

TABLE 14

Serum Glutamic Oxalacetic Transaminase Values in Sheep Injected with Adenia Extract

Day of injection	SGOT (Sigma-Frankel units/ml)				
	279	274	302	298*	271*
1	18	20	20	16	18
2	20	24	24	27	30
3	20	18	20	20	18
4	24	30	30	27	22
5	27	30	27	30	30
6	30	27	30	30	24
7	30	34	30	30	30
8	40	46	54	34	30
9	56	60	58	46	40
10	56	58	60	43	40
11	50	56	56	34	43
12	50	66	50	34	38
13	48	60	60	44	42
14	50	52	60	43	34
15	43	49	60	40	38
16	56	69	46	38	38
17	50	-	56	44	50
18	56	-	-	38	45
19	46	-	-	40	48
20	50	-	-	42	56
21	56	-	-	43	43
22	-	-	-	44	43
23	-	-	-	42	48

* Controls

TABLE 15

Serum Glutamic Pyruvic Transaminase Values in Sheep Injected With Adenia Extract

Day of injection	SGPT (Sigma-Frankel units/ml.)				
	279	274	302	298*	271*
1	34	44	44	42	44
2	34	40	42	36	42
3	40	44	36	42	38
4	36	48	42	42	42
5	42	42	42	40	36
6	-	-	-	-	-
7	40	32	28	38	42
8	24	22	28	32	34
9	28	24	28	30	42
10	34	32	36	38	38
11	22	34	32	36	42
12	28	30	40	32	40
13	24	28	38	40	40
14	34	28	36	42	38
15	38	22	38	40	36
16	36	38	36	32	34
17	40	-	36	38	42
18	36	-	-	32	40
19	32	-	-	36	38
20	22	-	-	34	36
21	32	-	-	36	42
22	-	-	-	28	28
23	-	-	-	38	30

* Controls

TABLE 16

Alkaline Phosphatase Levels in Sheep injected With Adenic Extract.

Day of injection	A.P. (King-Armstrong Units/100 ml.)				
	279	274	302	298*	271*
1	8	9	13	12	8
2	8	13	11	11	12
3	8	10	11	8	9
4	10	12	13	9	8
5	8	11	9	10	9
6	9	14	10	12	10
7	8	12	10	11	9
8	11	10	10	12	9
9	9	11	8	10	11
10	12	---	9	13	11
11	10	9	11	12	10
12	7	10	10	9	10
13	9	13	9	9	11
14	7	11	14	9	10
15	8	10	13	13	9
16	8	8	11	14	8
17	11	-	10	10	11
18	10	-	-	11	10
19	8	-	-	9	12
20	9	-	-	11	8
21	9	-	-	12	9
22	-	-	-	14	10
23	-	-	-	11	10

* Controls

Fig. 41. Rat poisoned with Adenia powder. Arrow shows diarrhoeic stool around the anal region.

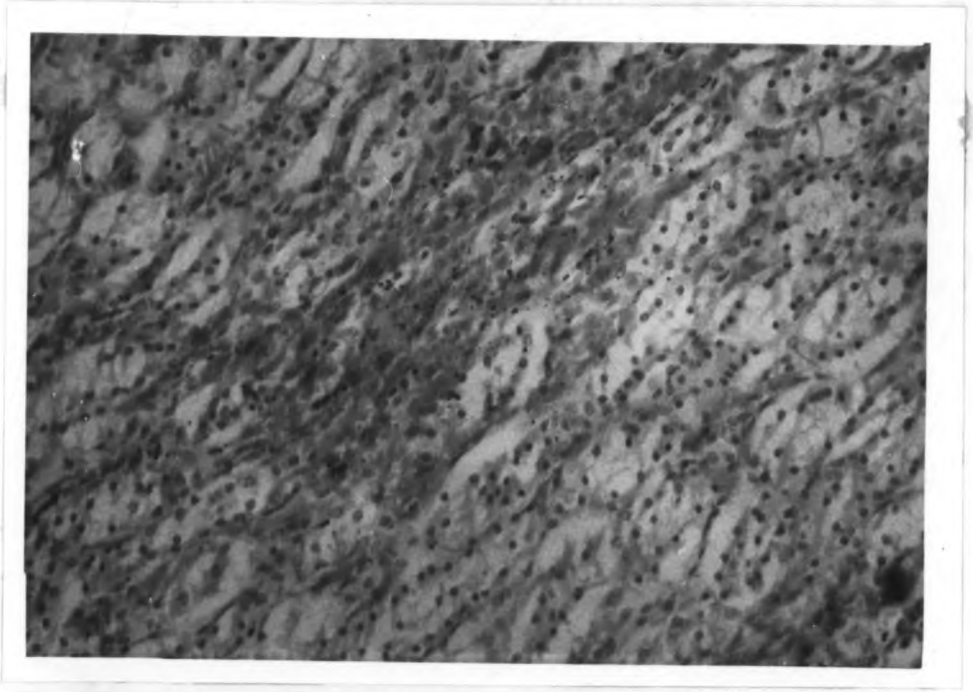

Fig. 42. Haemorrhage in the kidney of a rat fed 40% Adenia powder for 5 days. H&E Stain: X 391.

Fig. 43. Haemorrhage. Section from spleen of Rat ADI fed 20% Adenia powder for 9 days. H&E Stain: X 156.

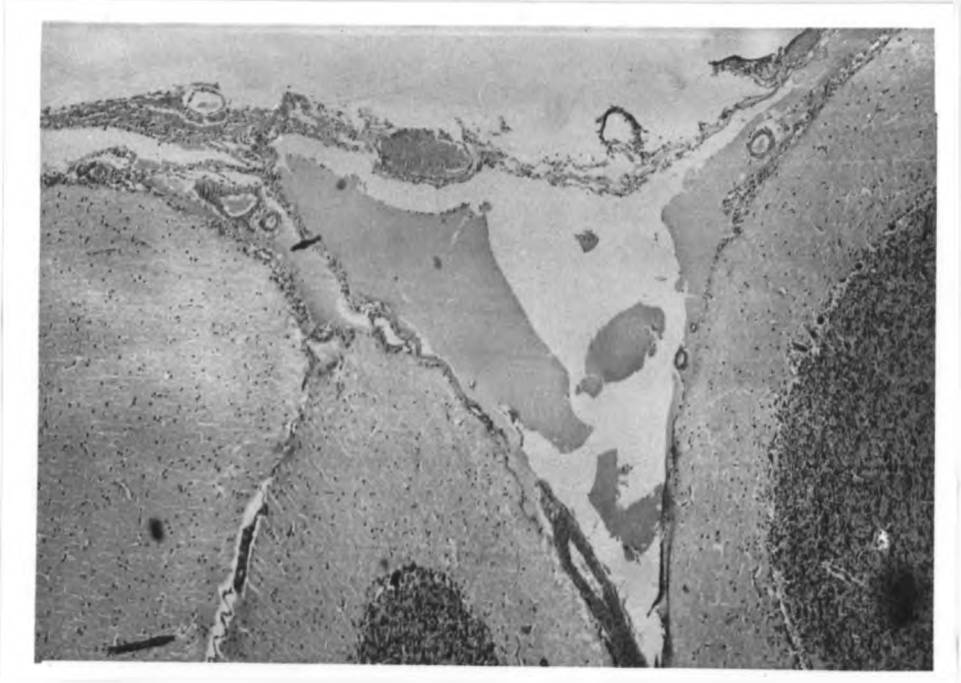

Fig. 44. Meningeal oedema of a rat fed 10% Adenia powder for 9 days. H&E Stain: X 156.

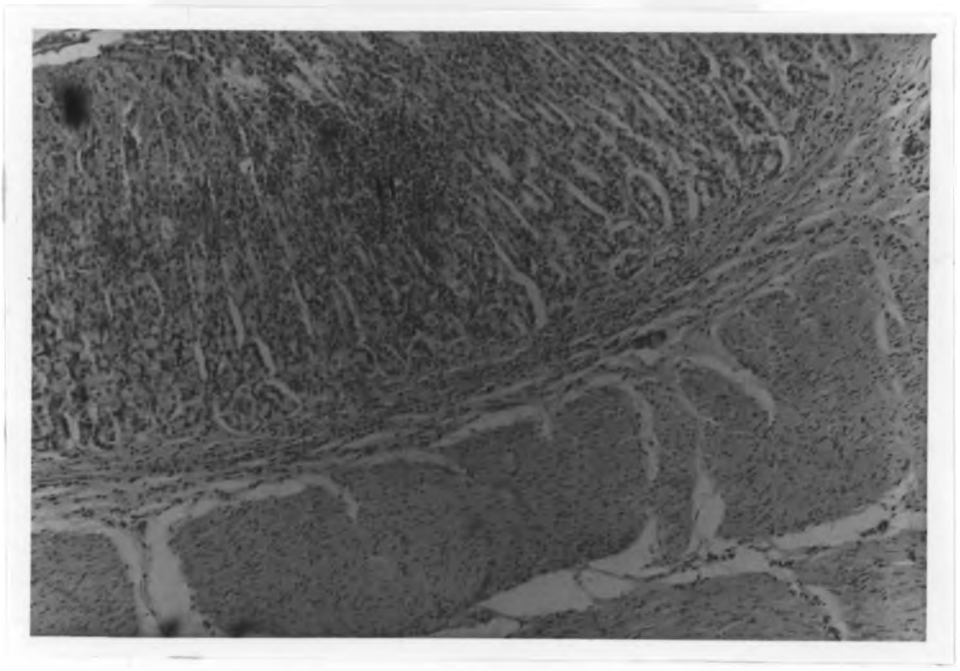

Fig. 45. Intestinal haemorrhage (H) rat fed 5% Adenia powder for 13 days. H&E Stain: X 156.

Fig. 46. Hyperaemia of the internal organs shown with arrow. Rabbit drenched Adenia extract, 3.3 ml./Kg. body weight.

Fig. 47. Epicardial haemorrhages (EH). Same rabbit as in Fig. 46.

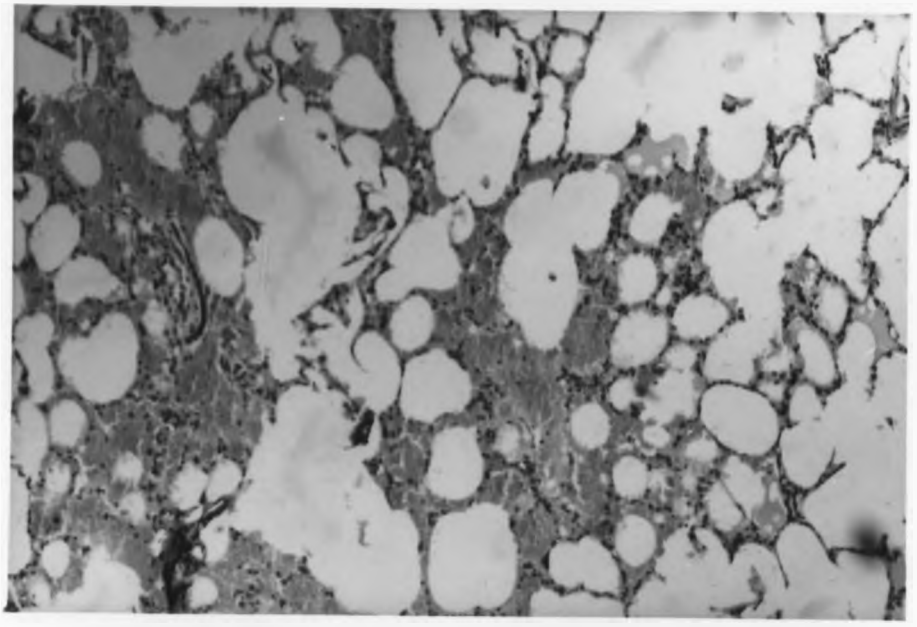

Fig. 48. Oedema, haemorrhage and emphysema. Lung section from rabbit drenched, 5.4 ml./Kg. body weight, Adenia extract. H&E Stain: X 156.

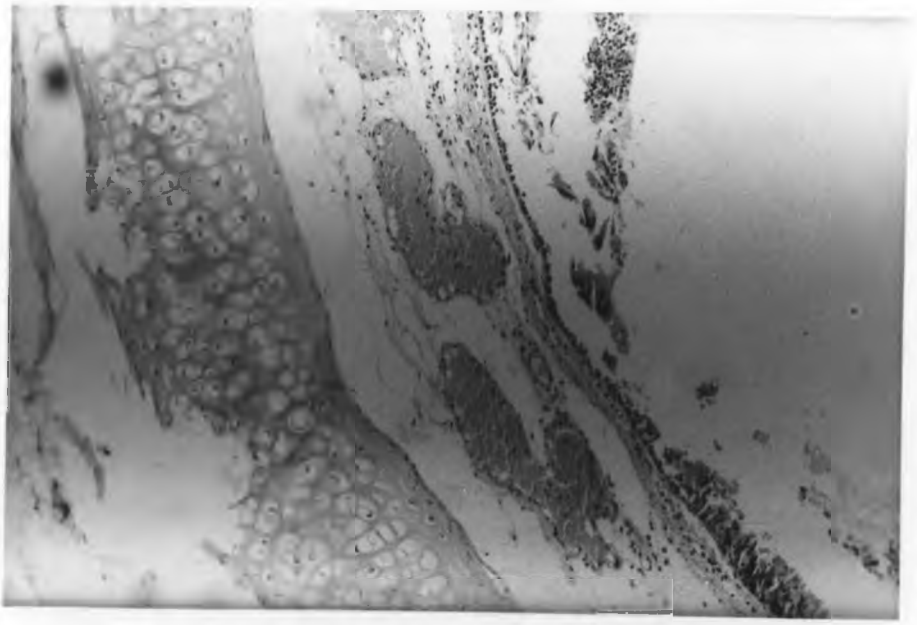

Fig. 49. Trachea. Section from a rabbit drench, 5.4 ml./Kg. body weight, Adenia extract. Oedema and Haemorrhage. H&E Stain: X 156.

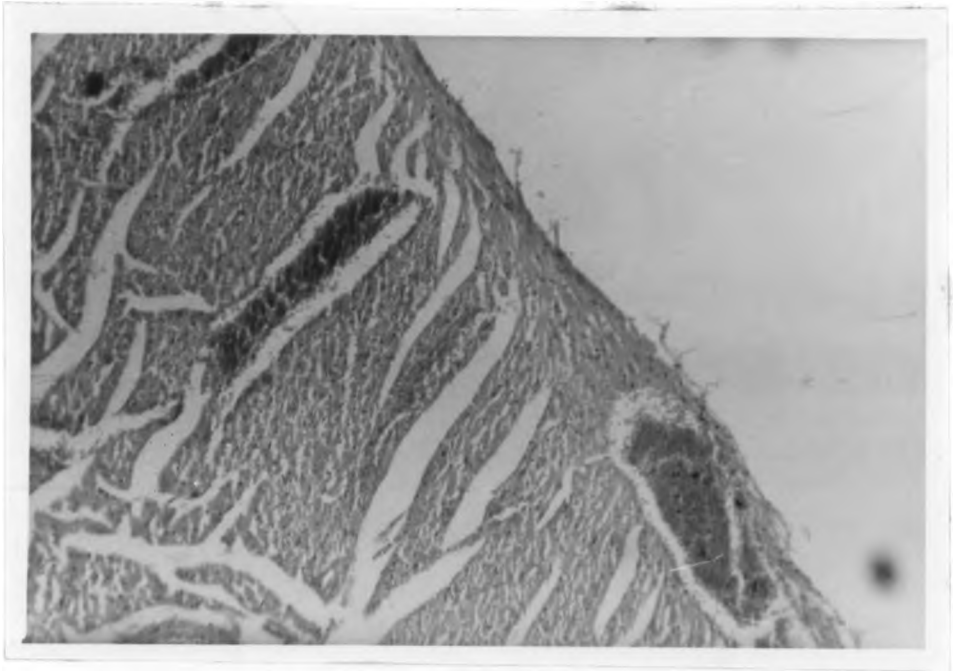

Fig. 50. Haemorrhage. Section from heart muscle of the same rabbit as in Fig. 49. H&E Stain: X 156.

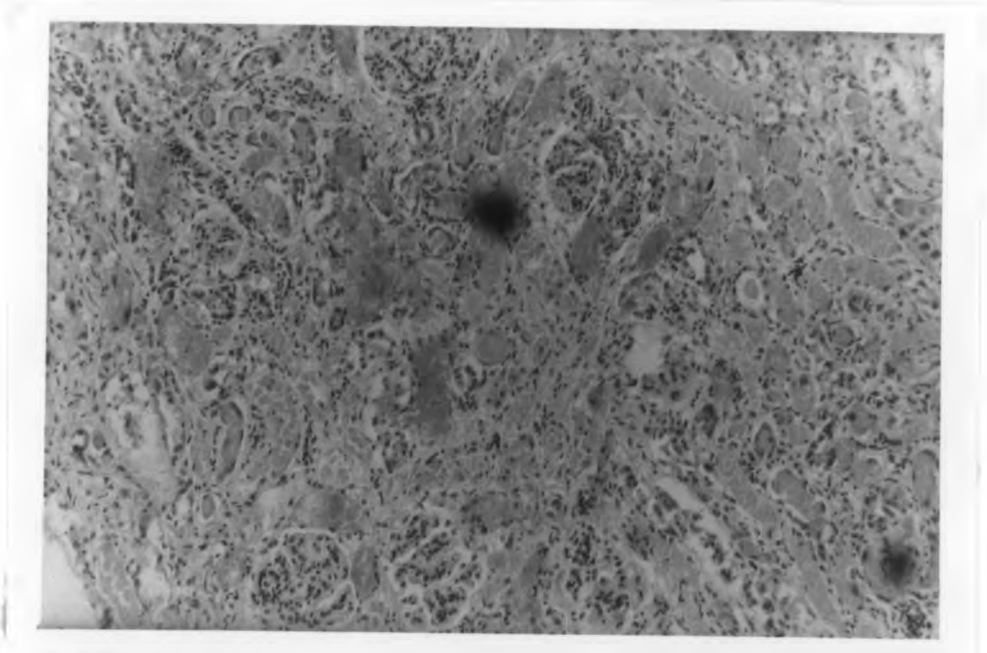

Fig. 51. Haemorrhage, tubular deposition of hyaline casts and necrosis of the kidney of a sheep injected, 0.03 ml./Kg. body weight, Adenia extract for 18 days. H&E Stain: X 156.

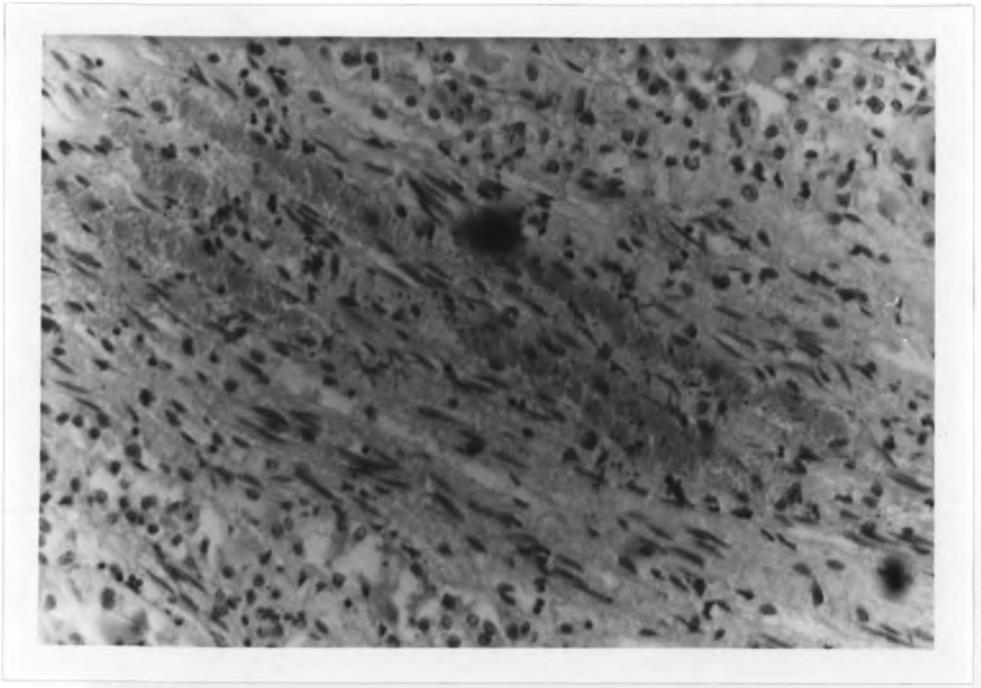

Fig. 52. Haemorrhage in the medulla of sheep's kidney. Sheep injected 0.01 ml/Kg. body weight Adenia extract for 22 days. H&E Stain: X 391.

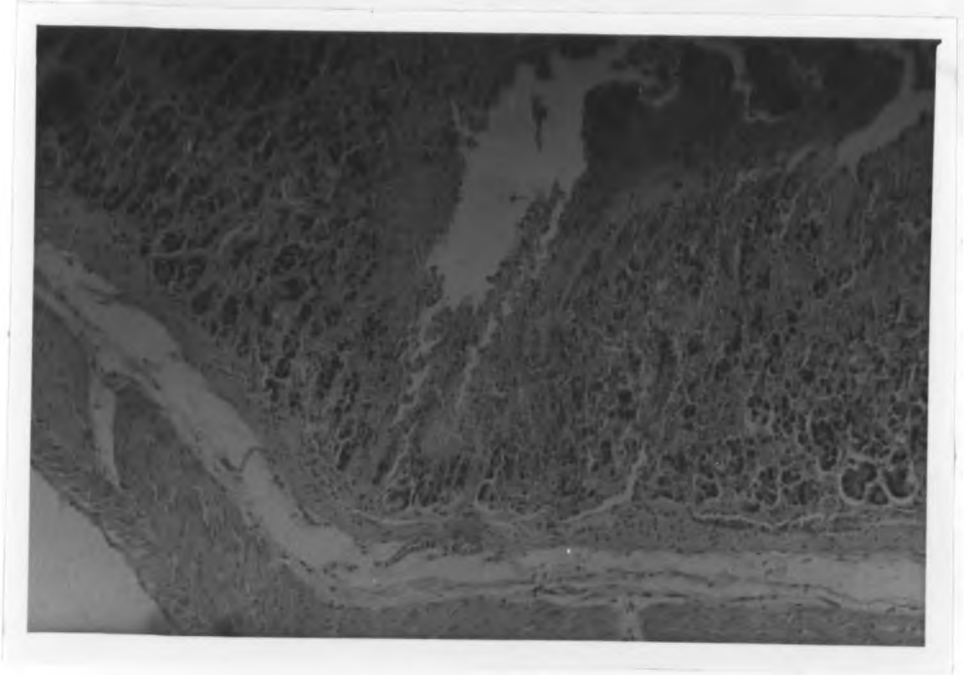

Fig. 53. Ulceration of the intestinal mucosa of the same sheep as in Fig. 52. H&E Stain: X 156.

Fig. 54. Oedema of the meninges. Same sheep as in Fig. 52. H&E Stain: X 156.

PART FOUR

PRELIMINARY INVESTIGATION OF CHEMICAL COMPOUNDS

PART IV PRELIMINARY INVESTIGATION OF THE CHEMICAL COMPOUNDS PRESENT IN:

(a) Senecio moorei R.E. Fries

INTRODUCTION

Drenching and feeding experiments conducted earlier in rats, cattle and pigs proved beyond doubt that Senecio moorei is toxic to these species. The lesions observed in these animals (particularly in liver) closely resembled those lesions produced by pyrrolizidine (Senecio) alkaloids poisoning reported in literature. It was therefore with the assumption that this plant might contain a similar alkaloid that the present investigation of the bases (alkaloids) acids and neutrals was conducted.

MATERIALS AND METHODS

Approximately one kilogram of ground Senecio moorei material (stem, twigs, leaves, flowers and seeds) was accurately weighed and placed in a clean pot for extraction. Eight litres of boiling water was added to the material and a well homogenised slurry obtained. This was acidified with 50% sulphuric acid, and left on a hot plate to simmer for approximately 6 hours. The aqueous extract was then strained through muslin cloth in a separating funnel. The residue was then taken up in approximately 4 litres acidified water and the extraction procedure repeated three times. The solution was then filtered using whatman No. 1

filter paper. To the combined filtrate, (approx. 500ml), lead acetate was added to precipitate the tannins. The precipitate was removed by centrifuging at 600 r.p.m. for about 3 hours. The supernatant was removed by decanting and the residue washed twice with small amount of water each time centrifuging the mixture for about 30 minutes. The excess lead ions were removed by adding 2N sulphuric acid, a few drops at a time swirlling the solution simultaneously, until there was no more precipitation. The white precipitate of lead sulphate was then removed by centrifuging or by filtration.

The clear detannated extract was evaporated to approximately 200 ml. and transferred to a 500 ml. separating funnel for solvent-solvent extraction.

Extraction Procedure

(1) Isolation of Acids and Neutrals

The detannated aqueous extract was checked for acidity and where necessary the pH was adjusted accordingly using N/10 sulphuric acid. The acidified extract was transferred to 500 ml. separating funnel and extracted with an equal volume of diethyl ether. During the extraction the immisible solvents were shaken gently so as to avoid emulsion formation and then left standing for approximately 12 hours. After 12 hours the ether extract was run off. The extraction of the aqueous

phase was repeated three times and the combined ether extract containing acids and neutrals washed with 2ml. N/10 sulphuric acid three times. The ether extract was then dried with anhydrous sodium sulphate and distilled off at 50°C in the usual distillation apparatus. The residue was then examined using thin layer chromatographic technique.

(2) Isolation of bases (alkaloids)

After the isolation of acids and neutrals the aqueous solution (layer) was made alkaline using 2N sodium hydroxide and then saturated with sodium chloride. The alkaline aqueous phase was then extracted with ether in the same manner as described above for acids and neutrals. The combined ether extract containing Senecio bases (alkaloids) was washed with 2ml. 2.5% sodium bicarbonate three times and the extract was then dried with anhydrous sodium sulphate. After filtration to remove anhydrous sodium sulphate ether was distilled off and the residue (a yellowish oily substance) examined by spot test and thin layer chromatographic technique.

EXAMINATION FOR BASES (ALKALOIDS) ACIDS AND NEUTRALS ISOLATED FROM SENECIO MATERIAL USING THIN LAYER CHROMATOGRAPHIC TECHNIQUE

(1) Bases (alkaloids)

(a) Spot Test for Alkaloids

The basic residue was spotted on a filter paper and Dragendorff's reagent was then applied on the spot. RESULT: An orange-yellow spot

indicated the presence of a base.

(b) Thin Layer Chromatographic Technique

The method used here was similar to that of Sharma et al., quoted by Bull et al. (1968). The Senecio basic residue was spotted on commercially prepared Thin Layer Chromatographic (T.L.C.) plate along with some known alkaloids and the plate developed in Chloroform: Methanol: Conc. Ammonia (85:14:1) Solvent System. The developing time was approximately 80 minutes and the Solvent System had travelled to almost half way the length of the plate (approx. 15 cm.). After drying the plate in air, it was first examined under ultraviolet light (short and long wavelengths). The plate was then exposed to iodine vapour, and the spots noted. Finally, after warming to get rid of iodine vapour, the plate was sprayed with Dragendorff's reagent and the spots noted again.

(2) Acids and neutrals

The acid-neutral residue was spotted on T.L.C. plates coated with Silica gel and developed in various solvent systems up to about halfway the length of the plates. The experimental procedure was as described for bases above. After drying in air the plates were exposed to iodine vapour. After experimenting with various solvents-systems Acetone: Chloroform (1:9) was chosen as this seemed to give the best resolution.

RESULTS

The results of the experiment are summarized in Figs. 55 and 56. As shown in Fig. 55 in all cases, three bases were revealed. The spot at the middle had a deep orange yellow colouration while the other two had a light yellow colouration. Fig. 56 show that in all cases, there was a streak of material extending from the base line to about $\frac{1}{3}$ of the distance travelled by the Solvent and another two spots on the upper half. The Rf values are also shown.

(b) Adenia volkensii Harms

INTRODUCTION

A literature survey has revealed that no work has been done to identify the toxic principle in this plant although its toxicity is generally well recognized, and the previous experiments had shown conclusively that it was undoubtedly poisonous. The purpose in the present study was, therefore, to identify and if possible estimate the major toxic principle in this plant.

MATERIALS AND METHODS

The material used in this experiment was collected at Kilungu Location, Machakos District, Eastern Province of Kenya and immediately put in plastic bags which were transported to the Faculty of Veterinary Medicine, Chiromo Campus and examined on the same day for the presence

of cyanide. The remaining material was stored in deep freeze until the next day when it was examined.

(a) Qualitative Determination of Cyanide

Approximately 500 g. of the fresh tubers, leaves and stems were minced and repeatedly extracted with cold(20-30°C) distilled water. Alternatively, the material was extracted with 80% ethanol and the combined extract was evaporated almost to dryness by blowing hot air current over the surface of the extract which was contained in a shallow porcelain dish. The residue was then taken up in water and the extract screened for cyanogenetic glycoside.

The method used for screening was that described by Feigl (1937). The reagent was prepared by mixing equal amounts of 3% copper acetate (Sol. I) and 1% benzidine in 10% acetic acid (Sol.II). Both solutions were prepared and mixed ex tempore and preserved in a test-tube. A small amount of test sample (extract) was put in a quickfit stoppered test tube and acidified with dilute H_2SO_4. A strip of filter paper was dipped in the reagent and waved slightly in the air to get rid of excess reagent. The moist paper was then exposed to the test sample contained in the stoppered test tube as shown in diagram I (Fig. 57), and then the test-tube warmed slightly.

(b) Quantitative Estimation of Cyanide in Fresh Material

A whole recently uprooted plant was selected and the tuber, stem and leaves were immediately separated and their total weights determined.

The analytical procedure followed was that described by Burns et al. (1970). 20g. sample of each part was weighed out accurately and homogenized with 600 ml. of 15% ethanol for approximately 5 minutes at a high speed and placed in screw-top quart jar. In each jar 20 mg. of emulsin was added to aid liberation of CN^- from the glycoside in the forage.

The jars were sealed with conventional Kerr lids, shaken vigorously and incubated at 30°C for 24 hr. Following incubation samples were mixed and filtered. Duplicate 150 ml. aliquots of the filtrate were obtained and transferred to Kjeldahl flasks and 80 ml. of HCN - ethanol mixture distilled into 125 ml. Erlenmeyer flasks containing 15 ml. of 2% KOH. Distillation was allowed to proceed slowly for at least 15 minutes when essentially all CN^- was believed to have distilled over. The distillates were transferred with several rinsings to 100 ml. volumetric flasks. Distilled water was used to bring to volume.

Twenty millilitres of the diluted distilate of each sample and 10 ml. of alkaline picrate solution (25g. anhydrous Na_2CO_3 and 5g. picric

acid, the later corrected for moisture, per litre) were pipeted into 100 ml. pyrex tubes (uniform in glass thickness and diameter). The tubes stoppered with cotton, were placed for exactly 5 min. in a water bath preheated to 94°C. The percentage transmittance of the final alkaline picrate solution was read on a spectrophotometer at 540 mu, (spectronic 20, Bausch and Lomb, Inc., Rochester, NY) and the cyanide concentration was determined from a standard curve also described by Burns et al. (1970) (See Fig. 58). Distillations of extracts of each sample were done in duplicate.

(c) Effect of Boiling Tissue on the Cyanide Content.

To determine whether or not boiling the fresh tissue resulted in any loss of cyanide, 20g. each of the bark and inner part were boiled in distilled water for 1 hour after which they were homogenised and analysed for cyanide as described above.

The experiment was repeated using other recently uprooted plants (chosen in random) collected from Machakos District.

RESULTS

(a) In the qualitative determination of cyanide, a deep blue colour developed on the filter paper indicating that the material contained a large concentration of cyanide.

(b) The typical analytical results for a single plant were as follows:-

TOTAL WEIGHT (GM.)

Leaves	46.3
Stem	64.9
Tuber (a) bark	159.6
(b) Inner part	429.1

As shown in TABLES 17 and 18, the plant material contained a large concentration of cyanide.

(c) EFFECT OF BOILING TISSUE ON CYANIDE CONTENT

As shown in TABLE 19, the figures in the last column as compared to those in TABLE 17 indicate that boiling of the inner part and the bark of the tuber for 1 hour drove out practically all the cyanide. This compares very well with the rats experimental findings (TABLE 7). When the experiment was repeated with other recently uprooted plants collected from the Machakos District, the cyanide concentration was always within the following limits: leaves, 120 to 145 mg. /Kg.; stem, 18 to 27 mg. /Kg.; tuber, 400 to 600 mg. /Kg. (bark) and 130 to 200 mg. /Kg. (inner part). The total amount of cyanide present in the tuberous rootstock was 90% to 95% of that found in the whole plant.

DISCUSSION FOR PART IV

Since the isolation of an alkaloid retrorsine from Senecio retrorsus by Manske (1931) a large number of plants belonging to the

genus senecio have been chemically investigated. Bull et al. (1968) described the simple alkaloid screening tests, based on precipitation reaction as not generally reliable when applied to detection of pyrrolizidine alkaloids. They recommended a laboratory extraction and assay by titration, even for preliminary examination of a screening nature.

In the present studies, Senecio moorei (Mau-Narok ragwort) was screened for the basic chemical compounds. The thin layer chromatographic technique employed in the present investigation has been used by other workers such as Henning and Sharma et al., both quoted by Bull et al. (1968). The results indicated that there are three alkaloids present in this plant. An attempt to purify the alkaloids was not successful. However, it was not the intention of the present work to identify fully the nature of the alkaloids in question although this calls for a detailed research in future.

Since the interest lay on the alkaloids, I did not go to the extent of isolating the acids from neutrals, but this separation is possible. The fact that tannic acid did not elute in the Acetone: Chloroform (1:9) Solvent system does not necessarily mean that the isolated components were not acids. Conclusions to this effect, however, could only be made by carrying out further work.

Many species of plants are known to contain hydrocyanic acid either free or more usually in form of a cyanogenetic glycoside, a compound

containing sugar and capable of yielding cyanide on hydrolysis (Garner, 1967). The cyanogenetic glycoside itself is nontoxic, but when brought into contact with appropriate enzyme (usually in vivo), it is decomposed and hydrocyanic acid liberated. In the intact plant, no such action takes place as it is only when the plant tissue is damaged or starts to decay that liberation of hydrocyanic acid begins.

From the present analysis it was possible to show that (1) Adenia **volkensii** (Kiliambiti) contains cyanide, either free or more likely as cyanogenetic glycoside, (2) the concentration of cyanide in the tuberous stock (about 90-95% of the entire plant) is of toxicological importance and (3) boiling of the tuberous stock drove out practically all the cyanide.

The concentration of chemical compounds vary considerably from season to season and from one geographical region to another (Van der Walt 1944). It was not the purpose of the present investigation to consider this variation fully as this would be a major project requiring much time and expense it has to be meaningful.

TABLE 17

QUANTITATIVE ESTIMATION OF CYANIDE IN ADENIA VOLKENSII

TISSUE	PERCENTAGE TRANSMITTANCE	CN^- CONTENT ug./20 ml	mg./Kg	Mean duplicate determination - mg./Kg.
Leaves	40	132	132	137
	37	142	142	
Stem	85	23	23	23
	85	23	23	
Tuber (bark diluted x 4)	37	142	568	568
	37	142	568	
Tuber (inner part diluted x 2)	57	85	170	170
	57	85	170	

SPECIMEN CALCULATIONS (e.g., leaves): Colorimeter reading (40% transmittance) corresponded to 132 ug. of CN^-/20 ml. of distillate; therefore, 100 ml. of distillate corresponded to 132 x 5 ug. of CN^-, but 100 ml. of distillate was from 150 ml. out of 600 ml. of original extract. Extract (600 ml., containing 20 Gm. of fresh leaves) contained 132 x 5 x 4 ug. of CN^-; therefore, cyanide concentration (mg./Kg.) of fresh tissue = (132x5x4/20) x (1000/1000) = 132 mg./Kg.

TABLE 18

TOTAL AMOUNT OF CYANIDE IN ADENIA VOLKENSII

TISSUE	CN^- CONTENT (mg.)
Leaves	6.343
Stem	1.493
Tuber (bark)	90.653
Tuber (inner part)	72.947
TOTAL	171.436

Of the total amount of cyanide present in Adenia volkensii (171.436 mg.), 163.6mg. (95.4%) was in the root; of the 163.6 mg. of cyanide present in the root, 90.653 mg. (55.4%) was in the bark.

SPECIMEN CALCULATIONS (e.g. leaves): total weight of leaves = 46.3 Gm., and concentration of cyanide = 137 mg. /Kg. of leaf; therefore, amount of cyanide in leaves = (137/1000) x 46.3 = 6.343 mg.

TABLE 19

EFFECT OF BOILING OF PLANT TISSUE ON CYANIDE CONTENT

PART OF TUBER	PERCENTAGE TRANSMITTANCE	CN^- CONTENT ug./100 ml.	CN^- CONTENT mg/Kg.	CN^- CONTENT Mean of duplicate determinations (mg./Kg.)
Inner part	95	3	3	3
	95	3	3	
Bark	94	5	5	5
	94	5	5	

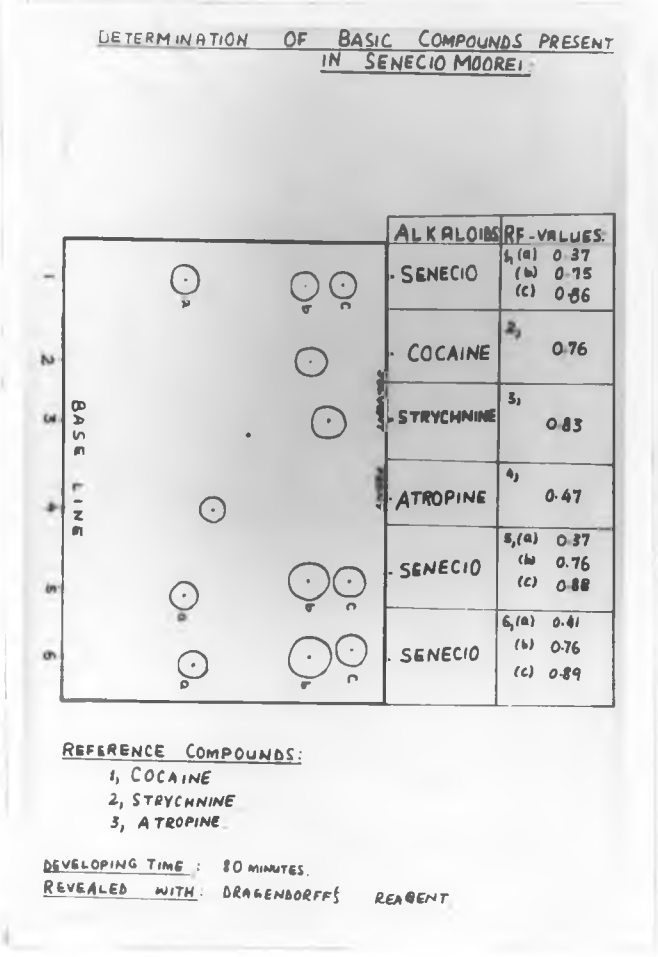

Fig. 55. Chromtogram of basic compounds present in Senecio moorei. R.E. Fries.

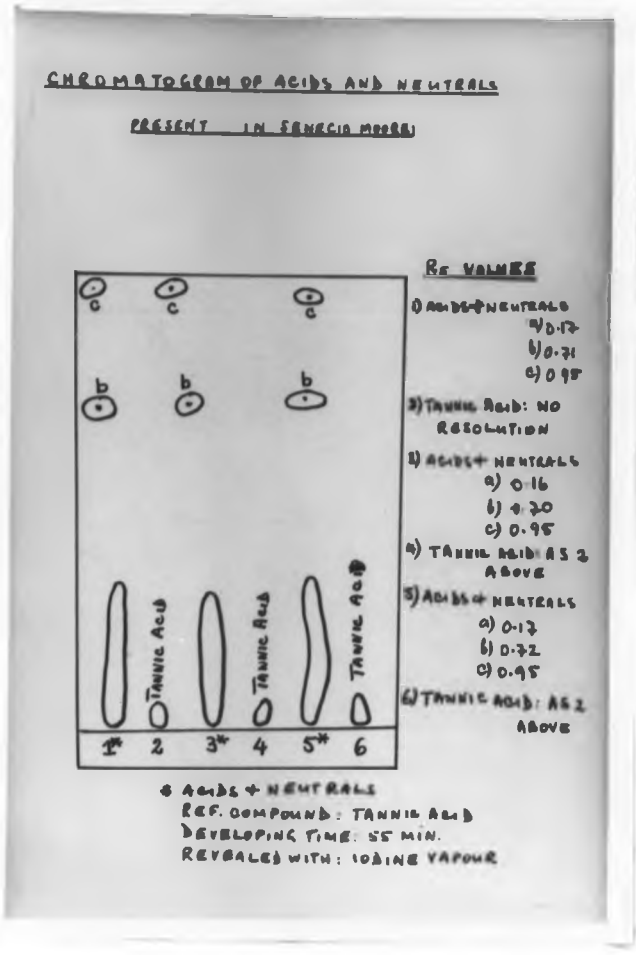

Fig. 56. Determination of Acids and Neutrals present in Senecio moorei R.E. Fries.

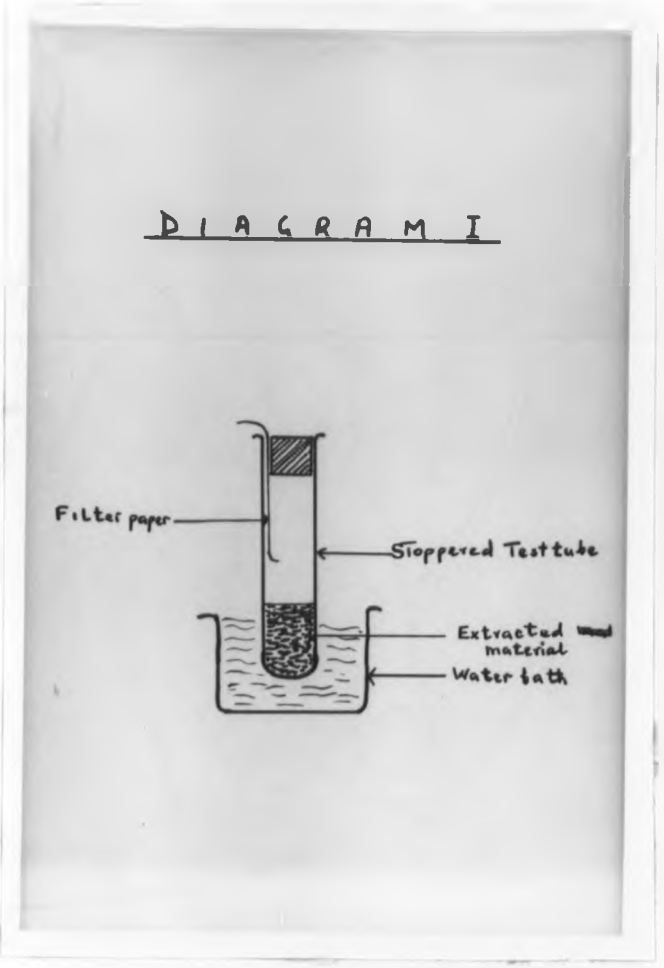

Fig. 57. Qualitative determination of cyanide in Adenia volkensii material.

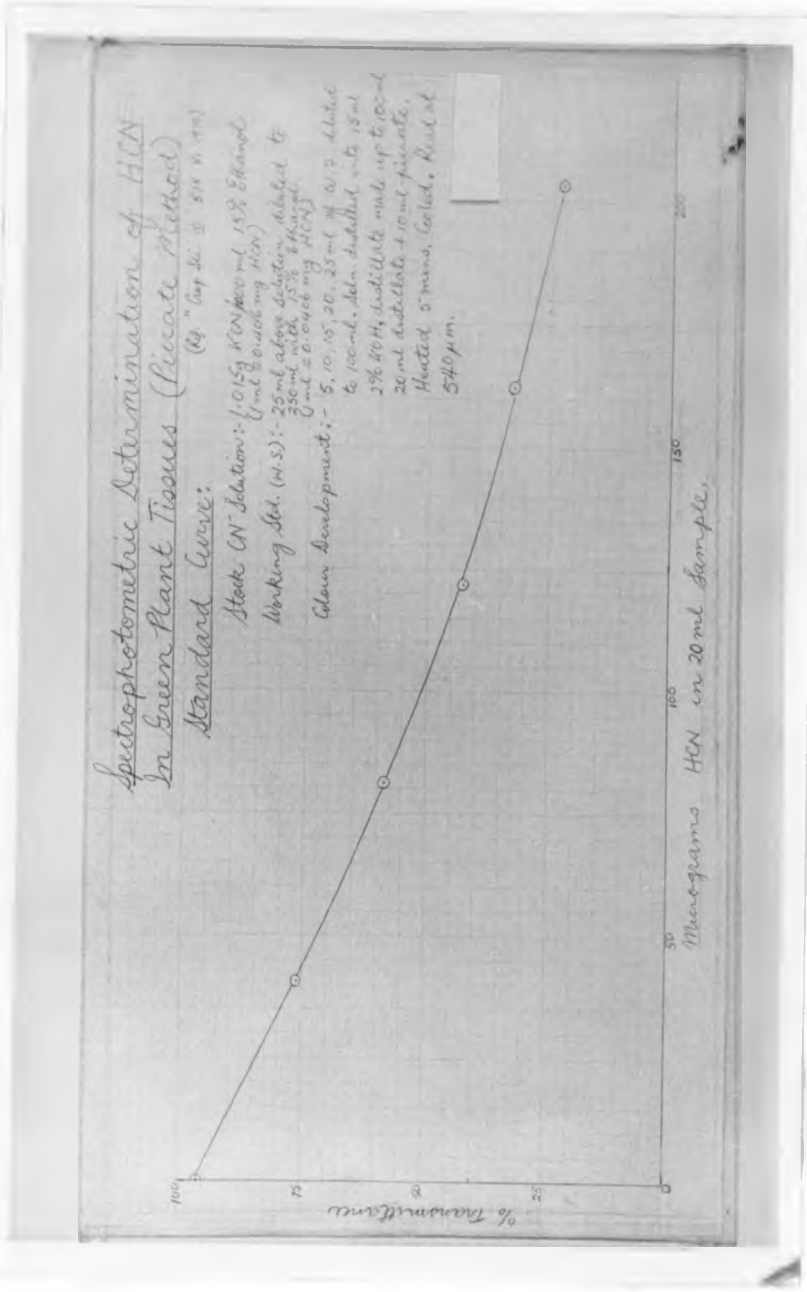

Fig. 58. Standard curve used in determination of cyanide concentration in Adenia volkensii.

GENERAL SUMMARY

Feeding and drenching Senecio moorei material to cattle swine and rats produced lesions in their livers, kidneys and other organs. The liver lesions in cattle and rats consisted primarily of portal and centrolobular fibrosis and degeneration, haemorrhage and necrosis respectively. The liver appeared to be more susceptible to the toxic substance than any other organ and the severity of the lesions was in proportion to the amount fed. The lesions in pigs were quite different from the rest of animals, the kidney having been more affected than the liver.

Tumours were produced in rats fed 1% Senecio powder for 13 months. The tumours were only in the liver and included, hepatomans, bile-duct adenomas and fibromas. These occurred either simultaneously or independently. In the chemical determination of the basic compounds present in Senecio moorei, three bases were isolated. It would appear that these basic compounds contributed to the toxic factor(s) of Senecio moorei. As the mechanism of Senecio alkaloids is not yet known, it has yet to be shown whether the hepatotoxic action was due to the basic compound(s) or their metabolic products.

The present experiments have shown conclusively that hydrocyanic acid either free or in form of cyanogenetic glycoside is one of the most important toxic factor(s) present in Adenia volkensii. All the experimental animals (sheep, rabbits and rats) treated with Adenia volkensii material produced lesions, most important of which were, degeneration, congestion and haemorrhages of the internal organs.

APPENDIX "A"

BLOOD CHEMISTRY IN BULL CALVES DURING THE EXPERIMENT

SGOT and SGPT (S-F. Units/ml.), T.P., ALB. and GLOB. (Gm./100ml.) A.P. (K - A Units/100 ml.)

WEEK	ANIMAL NO.	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALB. GLOB.	STOT	SGPT	ALKALINE PHOSPHATASE
1	6540	6.4	3	3.4	0.9	67	23	5.4
	6580	5.7	3.2	2.5	1.3	65	25	4.5
	6686	5.7	2.7	3.0	0.9	66	26	4.5
	6688	6.2	2.9	3.3	0.9	51	26	4.0
2	6540	6.6	3.6	3.0	1.2	60	27	4.5
	6580	6.4	3.0	3.4	0.9	66	26	5.1
	6686	5.5	2.4	3.1	0.8	59	23	4.0
	6688	5.6	3.1	2.5	1.2	60	30	4.2
3	6540	5.4	2.7	2.7	1.0	61	23	5.0
	6580	5.8	2.9	2.9	1.0	60	23	4.0
	6686	4.7	2.3	2.4	0.9	62	21	5.5
	6688	5.4	2.3	3.1	0.7	66	23	4.5
4	6540	6.4	2.7	3.7	0.8	68	21	5.5
	6580	6.6	3.1	3.5	0.9	66	26	4.5
	6686	5.3	2.3	3.0	0.8	46	21	4.5
	6688	5.9	2.7	3.2	0.8	67	22	5.5
5	6540	6.1	2.9	3.2	0.9	41	32	6.5
	6580	6.1	2.3	3.8	0.6	38	32	5.5
	6686	4.7	2.1	2.6	0.8	41	28	5.0
	6688	5.9	2.7	3.2	0.8	41	28	5.0
6	6540	6.0	3.0	3.0	1.0	69	28	5.6
	6580	5.7	2.9	2.8	1.0	66	23	6.8
	6686	5.4	2.5.	2.9	0.9	60	26	6.9
	6688	6.2	2.7	3.5	0.8	60	23	5.0

APPENDIX "A" Contd.

7	6540	6.0	2.9	3.1	0.9	64	24	6.4
	6580	5.7	3.0	2.7	1.1	70	26	5.9
	6686	6.2	2.3	3.9	0.6	62	21	4.5
	6688	5.3	2.3	3.0	0.8	62	21	6.8
8	6540	6.6	3.1	3.5	0.9	62	26	5.5
	6580	6.0	3.0	3.0	1.0	60	28	4.5
	6686	6.6	2.8	3.8	0.7	66	16	4.5
	6688	5.2	2.6	2.6	1.0	66	28	5.4
9	6540	6.1	2.7	3.4	0.8	68	21	4.5
	6580	6.0	3.0	3.0	1.0	60	22	4.8
	6686	4.7	2.3	2.4	0.9	67	24	4.6
	6688	5.0	2.4	2.6	0.9	68	26	5.2
10	6540	6.0	1.4	4.6	0.3	62	23	4.1
	6580	6.4	3.0	3.4	0.9	68	18	5.8
	6686	5.5	2.2	3.4	0.7	62	20	5.7
	6688	5.6	1.3	4.3	0.3	70	20	5.7
11	6540	6.1	1.2	4.9	0.2	98	37	4.5
	6580	6.4	3.0	3.4	0.9	68	2.8	5.5
	6686	5.6	2.2	3.4	0.7	62	31	3.9
	6688	5.6	1.5	4.1	0.4	124	31	4.5
12	6540	6.0	0.8	5.2	0.15	124	26	4.7
	6580	5.8	2.7	3.1	0.9	70	38	5.5
	6686	5.8	2.8	3.0	0.9	68	38	3.8
	6688	5.6	1.1	4.5	0.2	140	31	4.5

APPENDIX "A" Contd.

13	6540	6.7	1.0	5.7	0.18	123	29	5.5
	6580	6.5	3.3	3.2	1.0	40	24	3.5
	6686	6.3	2.9	3.4	0.85	44	34	4.5
	6688	6.2	0.6	5.6	0.1	156	40	3.0
14	6540	6.4	1.2	5.2	0.2	149	27	5.5
	6580	6.2	2.9	3.3	0.9	52	27	4.0
	6586	6.0	2.3	3.7	0.6	64	18	4.5
15	6540	6.4	1.2	5.2	0.2	156	21	4.1
	6580	6.9	3.0	3.9	0.8	60	24	3.9
	6586	6.9	2.4	4.5	0.5	68	24	3.7
16	6540	5.6	1.3	4.3	0.3	138	39	4.9
	6580	5.8	2.3	3.5	0.6	70	28	3.9
	6586	6.7	2.8	3.9	0.7	69	24	4.7

APPENDIX "B"

BLOOD PICTURE OF BULL CALVES FED WITH SENECIO MOOREI.

WEEK	CALF	RBC 10^6	PCV%	MCV U^3	WBC 10^3	HB Gm%	N%	E%	L%	M%	B%
1	6640	6.46	39.7	48	13.9	13.7	19	1	81	-	-
	6580	7.26	29.6	40	14.2	10.7	33	-	66	-	-
	6686	5.20	13.4	46	5.5	8.2	18	2	80	2	-
	6683	7.27	29.9	42	8.0	11.3	18	2	81	2	-
2	6540	9.74	43.3	46	16.4	16.5	43	2	55	-	-
	6580	8.21	34.9	43	10.7	12.6	17	-	93	-	-
	6686	5.38	24.2	,6	7.0	9.6	16	-	84	-	-
	6688	7.47	32.2	44	9.4	12.1	18	-	81	1	-
3	6540	7.59	32.7	44	13.7	13.2	28	-	90	2	-
	6580	7.27	30.1	42	12.8	11.6	19	-	81	-	-
	6686	5.23	25.6	49	6.2	8.3	10	1	89	-	-
	6688	6.64	27.5	42	9.8	10.2	24	-	76	-	-
4	6540	8.74	38.9	45	10.5	12.8	23	3	73	1	-
	6580	8.42	34.8	42	11.0	11.3	10	-	90	-	-
	6686	4.91	22.8	47	7.5	7.3	20	-	80	-	-
	6688	6.84	26.3	39	7.6	9.5	22	-	76	2	-
5	6540	8.81	39.2	45	11.3	12.3	28	1	69	2	-
	6580	7.19	29.9	42	10.5	9.3	15	2	83	-	-
	6586	4.13	19.3	47	4.7	6.6	16	2	80	2	-
	6688	7.22	28.1	40	4.4	9.1	26	-	74	-	-
6	6540	7.81	35.9	44	11.2	12.8	19	-	81	-	-
	6580	7.18	30.3	43	12.5	10.3	15	-	82	3	-
	6686	4.76	23.4	46	5.4	7.7	19	1	79	-	-
	6688	6.58	28.0	41	7.8	9.5	21	-	79	-	-
7	6540	7.32	32	48	13.5	12.2	21	1	78	-	-
	6580	6.95	30.2	44	11.0	10.5	24	-	71	5	-
	6686	4.66	23.3	50	5.6	7.4	18	-	83	-	-
	6688	6.52	28.4	44	6.8	9.7	20	-	80	-	-

APPENDIX "B" Contd.

8	6540	7.32	34.1	47	13.2	13.0	25	2	72	1	-
	6580	7.18	31.6	45	11.7	11.4	20	-	78	2	-
	6686	5.16	26.6	52	6.5	9.1	15	1	84	-	-
	6688	7.19	30.8	44	6.9	10.2	12	-	88	-	-
9	6540	7.58	33.4	45	11.5	11.5	17	4	79	-	-
	6580	7.73	34.2	45	13.4	11.9	14	4	82	-	-
	6686	5.43	26.0	48	6.4	9.1	11	5	84	-	-
	6688	6.23	26.5	43	11.9	9.8	10	4	86	-	-
10	6540	7.71	37.8	50	13.3	12.6	22	6	72	-	-
	6580	7.76	33.7	44	12.8	12.0	32	4	64	-	-
	6686	5.77	28.1	49	6.5	9.7	26	5	69	-	-
	6688	6.57	30.4	47	8.5	10.2	15	4	81	-	-
11	6540	6.98	35.5	51	12.7	11.3	14	4	82	-	-
	6580	7.76	32.8	43	12.3	11.7	18	5	77	-	-
	6686	5.83	28.5	49	9.2	9.5	23	7	70	-	-
	6688	7.11	30.1	43	7.7	10.7	15	5	80	-	-
12	6540	8.00	40.4	51	13.8	13.5	18	5	77	-	-
	6540	8.71	41.2	48	14.9	13.0	20	4	76	-	-
	6686	6.38	30.2	48	7.4	10.3	14	3	83	-	-
	6688	7.02	30.4	44	16.1	11.1	13	4	83	-	-
13	6540	8.27	38.7	48	14.6	12.0	14	4	82	-	-
	6580	7.99	33.6	43	12.5	12.3	15	5	80	-	-
	6686	6.01	29.0	49	7.2	7.7	16	1	83	-	-
	6688	6.73	30.3	46	7.5	10.3	16	4	79	-	-
14	6540	7.16	32.5	46	12.9	11.5	10	1	79	9	-
	6580	7.24	30.4	43	10.4	11.0	18	2	74	1	-
	6586	5.42	25.6	48	6.3	8.9	24	2	79	-	-
15	6540	6.14	30	50	7.3	11.7	18	1	81	-	-
	6580	6.99	26	37	10.8	10.1	20	2	75	-	-
	6686	4.89	24.5	50	6.4	9.9	19	1	78	-	-
16	6540	7.40	30	41	11.0	11.2	14	2	77	-	-
	6580	6.89	26	38	11.8	10.0	15	3	71	-	-
	6686	5.85	27	42	6.8	9.2	17	3	80	-	-

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