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Carcinogenic and Mutagenic Activity of an Alkaloidal Extract of *Senecio nemorensis* ssp. *fuchsii*

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Dedicated to Professor Dr. Horst Habs on the occasion of his 80th birthday

Summary: The alkaloidal residue obtained after extraction of dried and pulverized *Senecio nemorensis* ssp. *fuchsii* contains two pyrrolizidine alkaloids, i.e. fuchsisenecionine and senecionine. In a chronic experiment doses of 8 mg/kg and 40 mg/kg b.w. of the alkaloidal extract were given five times a week by gavage to male and female Sprague-Dawley rats. The study was terminated after 114 weeks. Repeated serum analysis demonstrated a dose-related elevation of serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (AP). Beside its hepatotoxic effect, the treatment proved to be hepatocarcinogenic, showing female rats to be more sensitive than males. Tumors diagnosed were of hepatocellular and angiogenic origin. In age-adjusted analysis the occurrence of these neoplasms revealed a clear dose-related trend ($p < 0.00005$). In the in-vitro mutagenicity test system with V 79 Chinese hamster cells, the alkaloidal extract exerted a weak but dose-related mutagenic activity. Under the conditions used the alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii* is a genotoxic carcinogen.

Zusammenfassung: Karzinogene und mutagene Wirkung eines Alkaloid-Extraktes aus *Senecio nemorensis* ssp. *fuchsii*

Key words: Fuchsisenecionine · Pyrrolizidine alkaloids · *Senecio nemorensis* ssp. *fuchsii*, alkaloidal extract, carcinogenicity, mutagenicity, toxicity · Senecionine

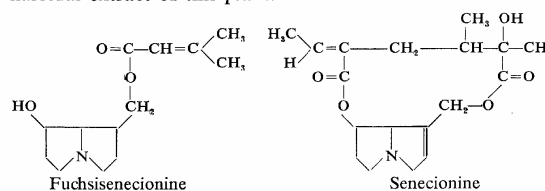
1. Introduction

Pyrrolizidine alkaloids are present in various plants of different botanical families [1]. They are often responsible for poisoning of livestock and sometimes of humans [2, 3, 4]. Extracts of plants containing pyrrolizidine alkaloids or the purified alkaloids as such have been reported to be toxic and carcinogenic in a variety of laboratory animal species, producing mainly damages and tumors in the liver [5-11]. Beside acute and chronic toxic effects, genotoxic activity of pyrrolizidine alkaloids has been observed in in-vitro and in-vivo mutagenicity test systems [12-15].

The alkaloidal residue obtained by extracting dried and pulverized *Senecio nemorensis* ssp. *fuchsii* contains the two pyrrolizidine alkaloids fuchsisenecionine and senecionine [16, 17].

Nach Extraktion der getrockneten und pulverisierten Droge *Senecio nemorensis* ssp. *fuchsii* erhält man einen Alkaloid-Extrakt, der die beiden Pyrrolizidinalkaloide Fuchsisenecionin und Senecionin beinhaltet. In einem chronischen Experiment wurden Dosierungen von 8 mg/kg und 40 mg/kg Körpergewicht des Alkaloid-Extraktes an männliche und weibliche Sprague-Dawley Ratten fünfmal pro Woche mit der Schlundsonde verabreicht. Die Studie wurde nach 114 Wochen beendet. Wiederholte klinisch-chemische Untersuchungen des Serums zeigten einen dosisabhängigen Anstieg der Serum-Glutamat-Oxalacetat-Transaminase (SGOT), der Serum-Glutamat-Pyruvat-Transaminase (SGPT) und der Alkalischen Phosphatase (AP). Neben dieser Hepatotoxizität führte die Behandlung zur Entstehung von Lebertumoren. Hierbei reagierten weibliche Ratten empfindlicher als männliche. Die beobachteten Tumoren waren hepatozellulären und angiogenen Ursprungs. Eine altersjustierte Analyse des Auftretens dieser neoplastischen Veränderungen zeigte einen eindeutigen dosisabhängigen Trend ($p < 0,00005$). In einem In-vitro-Mutagenitätstestsystem mit V 79 Chinesischen Hamsterzellen entwickelte der Alkaloid-Extrakt eine zwar schwache, aber dosisabhängige mutagene Wirkung. Der Alkaloid-Extrakt aus *Senecio nemorensis* ssp. *fuchsii* erwies sich unter den Versuchsbedingungen als ein genotoxisches Kanzerogen.

On the German market a few (about 4) drugs prepared of dried *Senecio nemorensis* ssp. *fuchsii* or extracts thereof are available. We therefore investigated the possible mutagenic activity and chronic toxicity of the phytochemical alkaloidal extract of this plant.



Chemical structures of the pyrrolizidine alkaloids of *Senecio nemorensis* ssp. *fuchsii*

2. Material and methods

2.1. Chemicals

The alkaloidal extract was prepared according to the literature [18a, b]. It was standardized to a content of 50% fuchsisenecionine and 1% senecionine by adding ethanol. It was dissolved in pure dimethylsulfoxide for the mutagenicity tests and in 16 vol. parts of ethanol (96%) and 84 vol. parts of a 1% aqueous solution of citric acid (pH 3) for the chronic experiment.

2.2. Mutagenicity experiments

V 79 Chinese hamster cells were used to determine a possible mutagenic activity of the alkaloidal extract. In this model the change from 8-azaguanine susceptibility to resistance is a marker for mutagenicity. The mutagenicity and cytotoxicity assays were carried out according to previously described methods [19]. The average plating efficiency of control discs was 100%. Mutagenicity was calculated per 10^5 survivors. The spontaneous background mutation rate in controls was 6 colonies/ 10^5 survivors. N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) served as a positive control substance. In addition the standard Salmonella typhimurium/mammalian microsome test was conducted according to the methods described by Ames et al. [20]. TA 1535, TA 1537, TA 1538, TA 98 and TA 100 were used as bacterial tester strains and routinely checked for efficiency as recommended [20]. Investigations were performed with and without metabolic activation by Aroclor 1254 induced rat-liver microsomes, containing an NADPH-generating system (S-9 mix).

2.3. Animal experiment

60 male and 60 female outbred Sprague-Dawley rats approximately 100 days old at the start of the experiment, were kept

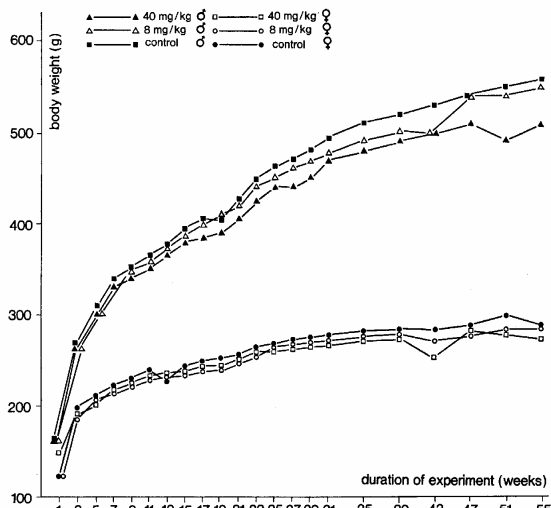


Fig. 1: Weight development of rats during the first 55 weeks in the chronic toxicity experiment with the alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii*.

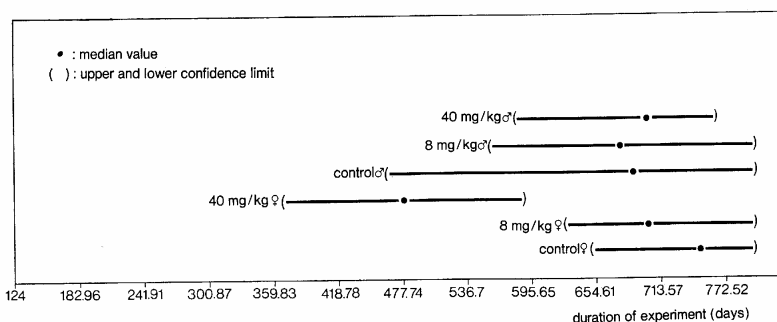


Fig. 2: Median survival times of rats and 95% confidence limits in the chronic toxicity experiment with the alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii*.

under conventional conditions [21]. The animals were stratified by sex and at random divided into 4 experimental and 2 untreated control groups of 20 rats each.

Food was withheld overnight, between 9 and 12 a.m. the alkaloidal extract was administered by gavage as an 8% or a 40% solution. Individual doses applied five times a week for 104 weeks corresponded to 8 and 40 mg/kg body weight. Subsequently the rats were given tap water and Altromin[®] pellets ad libitum. Animals found moribund and rats still alive after 114 weeks were killed. All rats were dissected. All livers and organs showing abnormalities were examined histologically. The tumor rates were analyzed statistically by comparing organ-specific, age-adjusted expected with observed incidences [22], reported p-values are one-tailed. Differences in survival times were analyzed using the methods of Wilcoxon et al., reported p-values are two-tailed [23].

Serum examinations were carried out in 2—8 animals/group every 8 weeks starting on week 68 after the beginning until termination of the experiment. Photometric determination included the following enzymes, SGOT, SGPT, AP and was carried out in the LKB Clinicon Kinetic Analyzer, Boehringer-Ultralab 8600. The concentrations of bilirubin and total protein in serum were determined by means of a digital photometer (Eppendorf PCP 6121). Standard assay procedures were carried out [24] using reagents from Boehringer Mannheim, FR Germany, readings of control sera (Precinorm U, Precipath U, Boehringer Mannheim) proved the tests systems to work correctly. The data obtained in the enzyme examinations were tested for dose-related trends [25].

3. Results

3.1. Mutagenicity experiments

The alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii* was weakly but dose-related mutagenic in cultures of V 79 Chinese hamster cells. The results are given in Table 1, showing mean values of three separate tests. In the standard plate incorporation test with *Salmonella typhimurium* the extract did not exhibit mutagenic activity tested from 0 to 100% cytotoxicity.

Table 1: Mutagenicity of the alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii* in cultured V 76 Chinese hamster cells.

Test substance	Concentration ($\mu\text{g/ml}$)	Plating efficiency (%)	Mutagenicity (revertants/ 10^5 survivors)
Dimethylsulfoxide (solvent control)	0.5%	100	6.1
MNNG (positive control)	0.20	59	87.3
	0.25	50	123.2
	0.50	26	167.2
	1.00	3	492.8
Alkaloidal extract	156.00	98	7.3
	312.00	86	7.9
	624.00	50	16.7
	1250.00	21	11.1

3.2. Animal experiment

During the first 55 weeks the weight development of animals of all groups was normal (see Fig. 1). Fig. 2 gives the median survival times including 95% confidence limits in the 4 treated and 2 control groups. Significant differences in survival times were established only when compared female animals of the high dose group with the female control group ($P < 0.01$) and between the two treated female groups ($P < 0.01$). The treatment with the alkaloidal extract resulted in a dose-related reduction of life expectancy in female animals.

The results of the enzyme examinations are presented in Figs. 3a—c. Determination of bilirubin and total serum protein did not reveal any difference between treated and control groups.

At all times of measuring concentrations of the enzymes AP (Fig. 3a), SGOT (Fig. 3b) and SGPT (Fig. 3c) were higher in male and female animals of the treated groups as compared to control animals. Statistical analysis demonstrated that the measured increased values of the serum enzymes are to be associated to treatment (in all cases: $P < 0.0005$). The results of the enzyme examinations suggested damages of the liver parenchymatous cells and, additionally, lesions of the bile duct in treated animals. Histological findings correlated with the results of the enzyme assays: In the liver fatty changes, single cell and focal necroses, fibroses, and granulomatous reactions were frequently detected.

The alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii* exerted a dose-related tumor-inducing effect in the liver

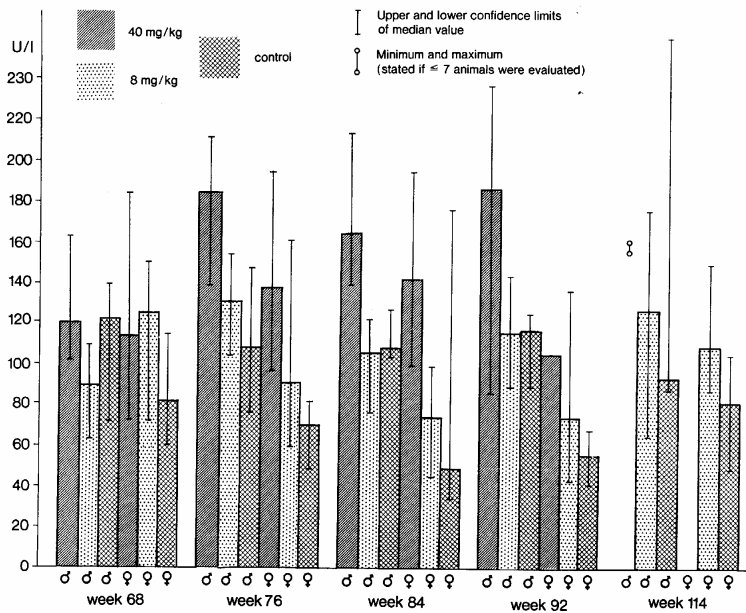


Fig. 3 a: Determination of alkaline phosphatase (AP) in serum in the chronic toxicity experiment with the alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii*.

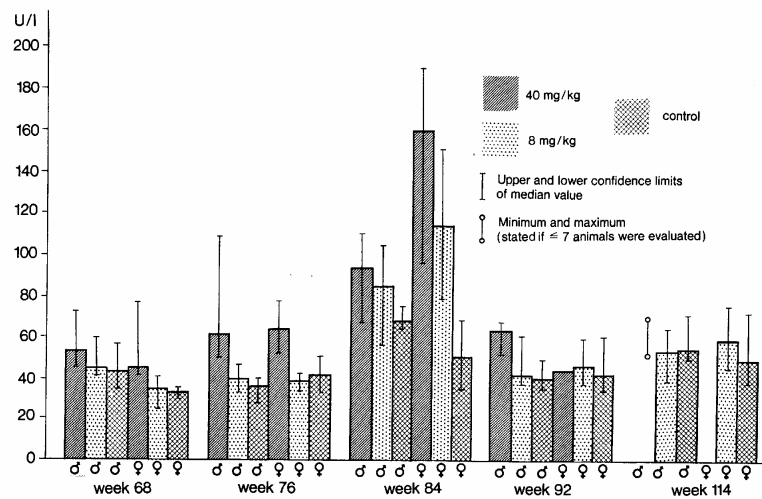


Fig. 3 b: Determination of serum glutamic oxalacetic transaminase (SGOT) in the chronic toxicity experiment with the alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii*.

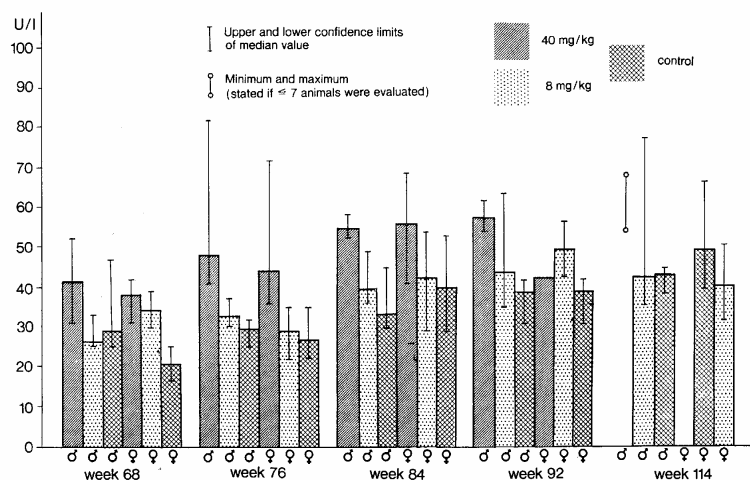


Fig. 3 c: Determination of serum glutamic pyruvic transaminase (SGPT) in the chronic toxicity experiment with the alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii*.

Table 2: Hepatocarcinogenicity of the alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii*.

Alkaloidal extract treatment (mg/kg b.w.)	Animals with neoplastic changes in the liver total*)					
	of hepatocellular origin		of cholangiogenic origin		of haemangiogenic origin	
	No.	%	No.	%	No.	%
Control ♂	1	5	1	5	0	0
8 ♂	2	10	1	5	1	5
40 ♂	5	25	1	5	2	10
Control ♀	0	0	0	0	0	0
8 ♀	11	55	4	20	2	10
40 ♀	29	145	13	65	11	55

*) If an animal had more than one histological type of tumor, each was counted separately.

(Table 3a, $P < 0.00005$). From Table 3b and c it is evident that female rats are more sensitive to the hepatocarcinogenic activity of the test substance. Histologic examination of specimens revealed that the following neoplastic changes occurred in the liver: hepatocellular, cholangiogenic and haemangiogenic tumors (Table 2).

It was only in femal animals of the high dose group that hepatocellular carcinomas were diagnosed which had form-

ed metastases and mostly caused death as stated during autopsy. In one male animal of the control group a hepatic neoplastic nodule was detected.

Table 4a lists benign and malignant extrahepatic tumors diagnosed in male animals of treatment and control groups. It is plausible to state that tumors diagnosed outside the liver in male animals were not treatment-related (Table 3d, $P > 0.2$), but occurred spontaneously.

Table 4b lists benign and malignant extrahepatic tumors diagnosed in female animals of treatment and control groups. The statement that the tumors diagnosed at other sites than the liver are treatment-related is of considerably lower probability of error in female rats as compared to males (Table 3e, $P < 0.025$). It therefore seems to be justified to classify the increased or earlier occurrence of extrahepatic neoplasms in females as probably substance-associated.

4. Discussion

Senecio nemorensis ssp. *fuchsii* has been applied as a drug since the Middle Ages. Even today the dried plant is used as a diabetic tea and plant extracts are administered systemically to treat gynaecological bleedings.

In our studies an alkaloidal extract of *Senecio nemorensis* ssp. *fuchsii* proved to be hepatotoxic and hepatocarcinogenic

Table 3: Organ-specific pooled, unbiased analysis of animals with neoplastic changes. Trend test statistics of observed versus expected incidence of tumor-bearing animals.

Organotropism	Alkaloidal extract treatment (mg/kg b.w.)	Observed value (o)	Expected value (e)	Ratio (o/e)	Statistical parameters			
					T	V	Z	P
a) Liver ♂ and ♀	0	1	20.45	0.05	973.57	7957.40	10.91	<0.00005
	8	13	19.11	0.68				
	40	34	8.44	4.03				
b) Liver ♂	0	1	2.47	0.40	79.96	1908.69	1.83	<0.04
	8	2	2.66	0.75				
	40	5	2.87	1.74				
c) Liver ♀	0	0	17.98	0.00	893.61	6048.72	11.49	<0.00005
	8	11	16.46	0.67				
	40	29	5.57	5.21				
d) All organs except liver ♂	0	4	4.93	0.81	51.63	4208.01	0.80	>0.2
	8	5	5.46	0.92				
	40	7	5.61	1.25				
e) All organs except liver ♀	0	6	7.17	0.84	71.37	1287.87	1.99	<0.025
	8	6	6.76	0.89				
	40	3	1.06	2.83				

Table 4 a: Benign and malignant extrahepatic tumors in male rats.

Organ System	Histological diagnosis	Alkaloidal extract treatment (mg/kg/b.w.)	Tumor latent period (d)
Testis	Leydigoma	0	637
	Leydigoma	8	645
	Leydigoma	40	742
	Leydigoma	8	802
Suprarenal gland	Pheochromocytoma	40	589
	Pheochromocytoma	0	680
	Pheochromocytoma	40	726
	Cortical adenoma	40	735
Subcutis	Hemangioendothelioma	40	667
	Cavernous hemangioma	8	802
Hypophysis	Chromophobe adenoma	8	787
Lung	Adenocarcinoma	0	802
Forestomach	Epithelial preneoplasma	40	735
Peritoneal cavity	Lipoma	0	474
Nervous tissue	Sarcoma	8	626
Hematopoietic and lymphatic system	Histiocytoma	40	409

Table 4 b: Benign and malignant extrahepatic tumors in female rats.

Organ System	Histological diagnosis	Alkaloidal extract treatment (mg/kg/b.w.)	Tumor latent period (d)
Mammary gland	Fibroadenoma	40	539
	Fibroadenoma	0	654
	Fibroadenoma	8	764
	Fibroadenoma	0	776
	Fibroadenoma	0	802
	Fibroadenoma	8	802
	Fibroadenoma	8	802
Ovary	Fibroma	8	631
	Thecoma	0	650
Hematopoietic and lymphatic system	Histiocytoma	40	584
	Histiocytoma	0	802
Velum	Squamous cell carcinoma	8	631
Forestomach	Epithelial preneoplasm	40	426
Uterus	Polyp	8	669
Nervous tissue	Sarcoma	0	616

in the rat. In reports on sex-linked biological effects of pyrrolizidine alkaloids, male animals were as a rule described to be more sensitive [8, 11, 26]. Contrary to these findings we observed a clearly higher sensitivity of female animals to the toxic and carcinogenic activities of the investigated extract. Since the extract of *Senecio nemorensis* ssp. *fuchsii* was also mutagenic in the in vitro bioassay with V 79 Chinese hamster cells, we have to conclude at present that the investigated substance is a genotoxic carcinogen. It is of interest to note that the results of the

standard Salmonella/microsome test in the case of this extract do not correlate to the in vivo carcinogenicity. This agrees with previous findings in which the value of the plate incorporation test for pyrrolizidine alkaloids has been questioned [12, 15, 27, 28]. Further detailed investigations will have to be carried out to specify the components which are responsible for the carcinogenic effect. The present limited investigation does not permit to make a risk extrapolation, for instance, by means of mathematical models which generally demand a well established dose-response relationship. Since however the extract of *Senecio nemorensis* ssp. *fuchsii* proved to be mutagenic in vitro and hepatotoxic and hepatocarcinogenic in animal experiments, it cannot be ruled out that it might be hazardous to man, too. Therefore, an individual risk-benefit estimate seems necessary. Before administering drugs prepared of *Senecio nemorensis* ssp. *fuchsii* the use of other drugs should be considered if these can be classified as less hazardous in their side effects and in particular their carcinogenicity, according to our present knowledge.

5. References

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