TOXICOLOGICAL STUDIES ON GINSENG

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ABSTRACT

Several publications in scientific journals affirming that ginseng could be toxic and have harmful effects on the organism, gave us reason to undertake extensive investigations as to the toxicity of the standardized ginseng extract G115, Acute and chronic toxicity, teratogenicity, carcinogenicity, its action on the cardiovascular and the hormonal system have been studied. The data obtained, which represent five years of research, are confirmed by clinical results:

The standardized ginseng extract G115 is absolutely safe, no toxic actions or side effects were observed.

INTRODUCTION

During the last years in scientific journals in Europe and the USA articles appeared tending to discredit Ginseng. These publications put doctors and the public on their guard from utilizing Ginseng and the preparations deriving thereof, because – at their point of view – it provokes toxic actions or very negative collateral actions.

The authors of these articles base their affirmations on very disputable scientific criteria and at the same time also demonstrate a great ignorance on the most elementary rules of chemistry and/or pharmacology.

Like this, Siegel (1979) confounds the structure of the ginsenosides with the one of digitalis glycosides and draws the conclusions that cases of euphoria and hypertension which he observed, are to ascribe to these substances. He does not only base his affirmations on psychiatric cases, but on drug users of substances other than Ginseng (dope). The type of Ginseng the patients have taken is not distinguished either: He confounds *Panax ginseng* with *Panax quinquefolium*, with Eleutherococcus senticosus and even with Rumex hymenosepalus. Not to talk about the dosages and galenic forms which have been used (even Ginseng cigarettes). Undoubtedly we can confirm that this unconirolled study is of little scientific value.

Palmer et al. 1978 describe a case of mastalgia observed in a 70-year-old woman who developed swollen, tender breasts after taking Ginseng powder. In this notice he doesn't mention origin and quality of the product.

1980 Punnonen and Lukola state that "Ginseng" has caused an oestrogen-like effect in a 62-year-old woman. The patient has shown these symptoms after ingesting "Rumanian ginseng". Leaving out the fact that we do not know a species called "Rumanian ginseng", also in this case the authors confound the triterpenic structure of dammarane-saponins with the one of oestrogenes!

The publications have been cited from authors who probably never in their lives even have seen a Ginseng root (Abramowicz 1980, Vaille 1982, Dayer 1983).

On the other side many products exist on the market which are supposed to contain *Panax ginseng* C.A. Meyer, but which really contain also other substances illegally added like aminopyrine, phenylbutazone (Ries and Sahud 1975), and other products which do not contain a trace of Ginseng (Liberti. 1978, Ruckert 1000).

As a company engaged in Ginseng research, we have – concerning the above said – considered it more than necessary to undertake a scientifically valid study, which must be as complete as possible, on the toxicology of our standardized Ginseng Extract G115 in order to eliminate any doubt still in existence.

The results which I present here very summarized in this short report are the fruit of 5 years of work carried out in various university centers, hospitals, pharmacological and toxicological laboratories in Europe and the United States.

Tables 1,2,3,4

Sex	Number of animals	Dose mg/kg	Number of dead animals	LD_{50} mg/kg
Male	20 20 20 20	2000 3000 4000 5000	0 0 0 0	>5000
Female	20 20 20 20	2000 3000 4000 5000	0 0 0 0	>5000

Table 1: Determination of LD_{50} in the mouse (administration per os)

Sex	Number of animals	Dose mg/kg	Number of dead animals	LD_{50} mg/kg
Male	20 20 20 20	2000 3000 4000 5000	0 0 0 0	>5000
Female	20 20 20 20	2000 3000 4000 5000	0 0 0 0	>5000

Table 3: Determination of LD_{50} in the rat (administration per os)

Sex	Number of animals	Dose mg/kg	Number of dead animals	LD_{50} mg/kg
Male	20 20 20	250 500 1000	0 0 0	>1000
Female	20 20 20	250 500 1000	0 0 1	>1000

Table 2: Determination of LD_{50} in the mouse (intraperitoneal administration)

Sex	Number of animals	Dose mg/kg	Number of dead animals	LD_{50} mg/kg
Male	20 20 20	250 500 1000	0 0 0	>1000
Female	20 20 20	250 500 1000	0 0 0	>1000

Table 4: Determination of LD_{50} in the rat (intraperitoneal administration)

RESULTS AND DISCUSSION

Acute Toxicity

Berte (1982) has investigated the acute toxicity of the standardized Ginseng Extract G115 after unique administration. The G115 was incorporated into a diet and administered to 280 mice, 280 rats ands6 mini pigs. The administered doses were 2,000mg, 3,000mg, 4,000mg, 5,000 mg of extract/kg body weight per os and 250mg, 500mg, 1,000mg of extract/kg I.P. for mice and rats. For the mini pig the dose was 2,000mg extract/kg per os. The time of observation was 14 days for mice and rats and 7 days for the mini pigs. The investigated parameters were mortality and state of health. For the mini pigs the state of health was evaluated following body weight, hematological and biochemical blood data. The results obtained for mice and rats sllow an LD_{50} of 5,000mg/kg by oral administration and 1,000 mg/kg by intraperitoneal administration.

G115 administered at the highest possible

doses (2,000mg/kg) per os, did not show any toxicological effect in the mini pig.

Sub-acute Toxicity

For 20 days the G115 was administered orally to rats at the dose of 4,000mg/kg per day (Savel 1971). Hematological examinations and histological structure of the organs of the animals were normal after treatment.

In conclusion it can be confirmed that the standardized Ginseng Extract G115 is a preparation devoid of acute or subacute toxicity effects.

Chronic Toxicity

Hess et al. (1982) have investigated the safety of G115 Extract on growth, reproduction, lactation and maturation of male and female rats through two successive generations.

1730 rats were used during a period of 33 weeks. The daily doses were of 0, 1.5, 5.0 and 15 mg/kg body weight and were administered orally.

The investigated parameters concerning reproduction and lactation of the treatment groups were comparable to those of the control group.

Table 5. Reproductive performance of two generations of female rats fed diets containing ginseng extract G115

Dietary level (mg/kg weight/day)	No. of mated	No. of pups per litter*		Indicest				Body weights of pups aged*			
										21 days	
	pregnant	Dorn alive	Born dead	Fertility	Gestation	Viability	Lactation	0 days	4 days	Male	Female
				F0 generation							
0	15(15)	11.9±0.6	0.2±0.1	100	100	98	98	6.3±0.1	10.8±0.3	50.5±1.2	46.9±1.3
1-5	15(13)	13.2±0.6	0.1±0.1	87	100	99	100	6.3±0.1	9.9±0.3	50.0±0.9	46.9±0.9
5	15(11)	12.0±0.6	0.2±0.2	73	100	100	99	6.2±0.1	10.5±0.3	49.3±1.2	47.0±1.2
15	15(13)	12.4±0.4	0.2±0.2	87	100	99	100	6.2±0.2	10.2±0.3	50.7±1.4	47.0±1.2
				F1 geseration							
0	30(29)	11.3±0.5	0.2±0.1	97	100	98	94	6.3±0.1	10.3±0.2	48.5±0.9	45.9±0.8
1-5	30(30)	11.7±0.4	0.4±0.2	100	100	97	96	6.1±0.1	10.2±0.3	50.2±1.0	47.1±0.8
5	30(28)	12.5±0.3	0.2±0.1	93	100	97	95	6.1±0.1	9.8±0.2	49.3±1.0	47.0±0.9
16	30(29)	11.9±0.4	0.1±0.1	97	100	98	93	6.2±0.1	9.7±0.3	50.8±0.9	47.4±1.0

* Values are means ± SEM for the number of litters or pups indicated.

† The indices were calculated as follows: fertility index–percentage of mated females that became pregnant: gestation index–percentage of pregnancies resulting in litters born alive: viability index–percentage of pups born alive that survived to 4 days of age; lactation index–percentage of pups alive at 4 days that survived to 21 days of age [each litter was called to eight pups (four males and four females for most litters) on day 4 post pertum].

There were no treatment-related effects observed for the male and female animals in regard to weekly body weights, food consumption, hematological, ophtalmological and clinical data, gross and histophathological examinations.

Hess et al. (1983) have investigated the safety of the G115 Extract in Beagle dogs. 32 animals were used during a period of treatment of 90 days at doses of 0, 1.5, 5.0 and 15 mg/kg daily.

Body weight, food consumption, absolute and relative organ weights, hematological and blood chemical parameters were examined using statistical methods. No treatment effects were seen.

At the end of the study all of the animals were sacrified with i.v. pentobarbital. The adrenal glands, brain, heart, kidneys, liver, pituitary gland, spleen, thyroid, epididymides and testes or ovaries and uterus of all animals were weighed. His topathological examination of control and high-dose animals encompassed the organs that were weighed and the following: eyes, gall bladder, large and small intestine, lungs, lymph

Fig. 1

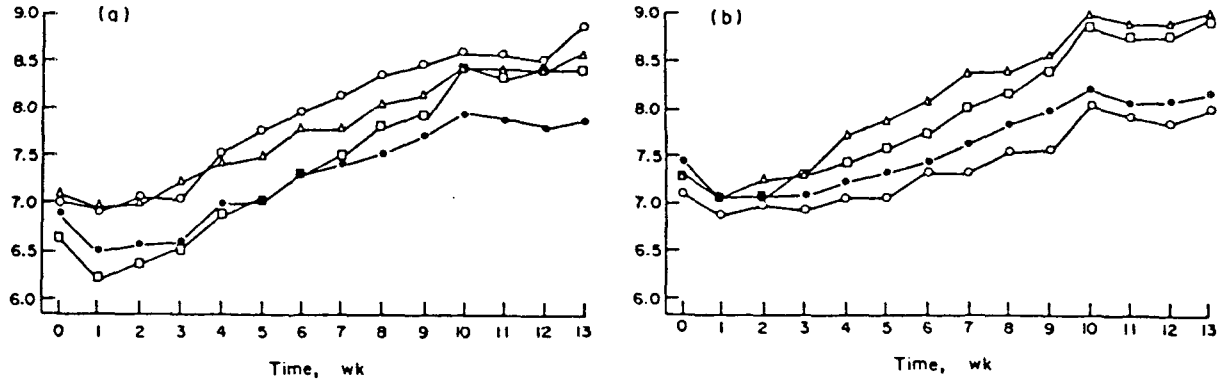


Fig. 1. Group mean body weights of (a) male and (b) female dogs fed ginseng extract G115 at a level of 0 (—●—), 1.5 (—○—), 5 (—△—) or 15 (—□—) mg/kg body weight for 13 wk.

nodes (mesenteric), mammary glands, pancreas, prostate, sciatic nerve, skeletal muscle, skin, spinal cord, sternal bone, marrow, stomach, urinary bladder.

No treatment related toxicity was observed in male and female dogs.

In conclusion it can be confirmed that the stardardized Ginseng Extract G115 at the dose of 15mg/kg body weight/day is a preparation devoid of chronic toxicity effects.

Teratology

In order to establish the absence of teratogenic effects, the G115 was administered to pregnant rats and rabbits. The rats were treated with 40mg/kg/day p.o. from the first to the 15th day after mating, and the rabbits received 20mg/kg/day p.o. from the 7th to the 15th day after mating. Their foetus' were extracted by caesarean section on the 21st day from the rats and on the 27th day from the rabbits.

Table 6. Tests of foetal development in Wistar rats, treated orally with 1 capsule (40mg of G115) per kg, from day 1 to day 15 of pregnancy

No. of animals		Group	Duration days	No. of foetuses		Resorptions	Mean weight of living foetuses	Mean living foetuses/ pregnant rat	Malformations
coverdd	impregnated			living	dead				
13	10	Controls	–	119	2	–	5.2	11.9	–
14	10	Test preparation	1 – 15	122	1	1	5.0	12.2	–

Table 7. Tests of foetal development in New Zealand rabbits, treated orally with 1/2 capsule (20mg of G115) per kg, from day 7 to day 15 of pregnancy

No. of animals		Group	No. of foetuses		Resorptions	Mean living foetuses/ pregnant rabbit	Malformations
covered	impregnated		living	dead			
9	6	Controls	33	1	–	5.5	–
8	6	Test preparation	30	3	2	5	–

Table 8. Genotoxicity in the hepatocyte-DNA repair test

Compound	Concentration	Results
Ginsenoside Rg_1	1 – 50µg/ml	negative (n=3)
G115 free of ginsenosides	0.1-10mg/ml*	negative (n=2)
G115	0.1-10mg/ml*	negative (n=2)
Total ginsenosides	0.1-10mg/ml*	negative (n=2)

* = cytotoxic at the highest concentration of 10mg/ml.

The standardized Ginseng Extract G115 given to pregnant animals produced no abnormality in foetal development.

Carcinogenicity Test

The Environmental Protection Agency (EPA) in the USA has published guidelines (Fed. Regist. 43, 163, 37388, 1978) for mutagenicity and carcinogenicity testing in the toxicity evaluation of drugs. These guidelines include the test which Althaus and Jenny (1984) have used to carry out this carcinogenicity test, i.e. the hepatocyte-DNA repair test which has the demonstration of primary DNA damage as an endpoing.

At the concentration tested, none of the extracts or ginsenosides scored positive in this carcinogenicity test.

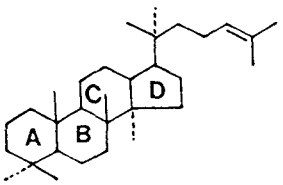
Oestrogen-Like and Gestagen-Like Effects

Steroid hormone receptors are very stereospecific (Kyrein 1976), i.e. the sterical structure

Fig. 2. Structural differences: Ginsenosides - Cardiac glycosides - Sexual hormons - Adrenocorticoid hormons

GINSENOSIDES

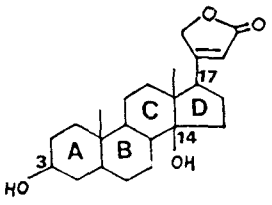
The Ginsenosides are triterpenic Saponins of the Dammaran-type.



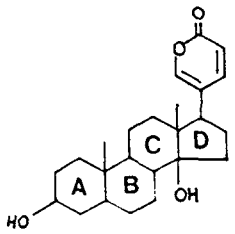
Conformation of the rings:

A/B trans (= chair) (= )
B/C trans (=chair)
C/D trans (= chair)

CARDIAC GLYCOSIDES

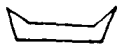


Cardenolide-type



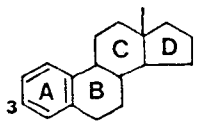
Bufadienolide-type

Conformation of the rings:

A/B cis (= boat) (= )
B/C trans(= chair)
C/D cis (= boat)

Necessary for the effect: β- Lactone ring at C-17
βOH at C-3 and C-14

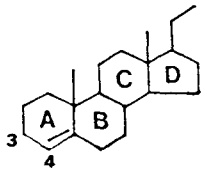
SEXUAL HORMONS



OESTRAN-DERIVATIVES

Conforamtion of the rings:
A/B cis
B/S trans
C/D trans

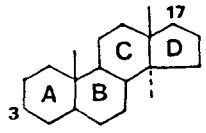
Necessary for the effect:
aromatic ring A
OH at C-3



PREGNAN-DERIVATIVES

Conformation of the rings:
A/B cis
B/C trans
C/D trans

Necessary for the effect:
double binding at C-4/C-5
usually =0 at C-3

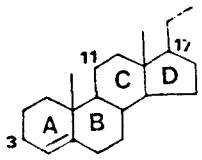


ANDROSTAN-DERIVATIVES

Conformation of the rings:
A/B trans
B/C trans
C/D trans

Necessary for the effect:
OH or =0 at C-17
OH or =0 at C-3

ADRENOCORTICOID HORMONS



PREGNEN-DERIVATIVES

Conformation of the rings:

A/B cis
B/C trans
C/D trans

Necessary for the effect:

GLUCOCORTICOIDS: OH or =0 at C-11
=0 at C-3
eventual OH t C D

eventual OH at C-17
MINERALOCORTICOIDS: =0 at C-3

(conformation) as well as functional groups of a "potential hormone" are of determining significance for the interaction with the receptor.

The ginsenosides have a completely different structure than sexual hormones, and adrenocorticoid hormones, therefore it is improbable that the ginsenosides can interact with these hormones.

The clinical trials of Forgo (1981) have proved that the standardized Ginseng Extract G115 does not cause a change in the male or female sex hormone status.

The examinations of Buchi and Jenny (1982, 1984) with the cytosolic oestrogen receptor of the mature rat uterus and with the progesterone receptors of the human myometrium show that.

Under physiological conditions an interference of the standardized Ginseng Extract G115 with hormone-receptors is not to be expected.

Cardiovascular Side Effects.

Because the structure of the ginsenosides is totally diferent to the one of cardiac glycosides, it is impossible that they exert side effects which are similar to the ones of the heart glycosides.

Jenny et al. (1982) have employed Gottingen mini-pigs to study the effect of G115 on the cardiovascular system. The following parameters were investigated: ECG, heart rate, blood pressure, cardiac output, stroke volume and (mathematically) the peripheral resistance.

Parenterally administered (i.v.) the ginsenosides have a proper effect on vessels which partly are concentration-dependent (dilatation or constriction).

Administered orally in doses of 0.250, 500 and 2,000mg/kg body weight, the G115 did not provoke any noticeable changes in the above mentioned parameters during the 6-hour observtion period.

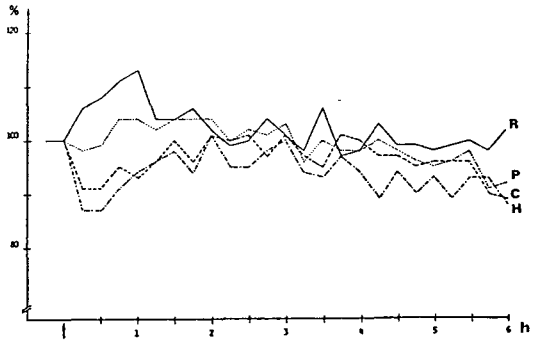


Fig. 3. Total peripheral resistance (R ————), blood pressure (P . . . . . .), cardiac output (C ---------) and heart rate (M ----------) in the untreated control group (n = 3)

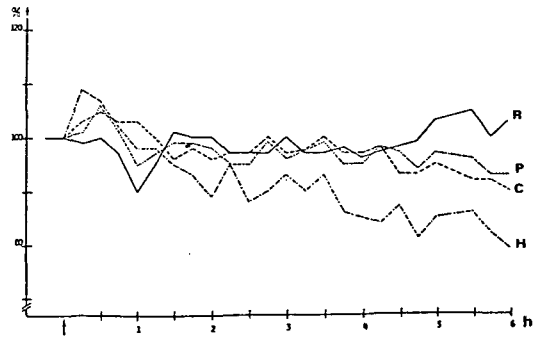


Fig. 4. Total peripheral reistance (K ———), blood pressure (P . . . . .), cardiac output (C ----------) and heart rate (M ----------) after 2g per os (per kg bodyweight) of the standardized Ginseng Extract G115 with 4: ginsenoside content (n = 3).

These results confirm the results obtained from Paik 1967 on frog hearts:

The standardized Ginseng Extract G115 does not provoke side effects on the cardiovascular system.

CONCLUSIONS

The results of the extensive studies on the standardized Ginseng Extract G115 performed in five kinds of animals (mouse, rat, rabbit, bedgle dog, mini pig) may be summarized as follows:

- Short term, medium term and long term studies failed to reveal any toxic effects.
- The oral LD_{50} is higher than 5g/kg body weight in mouse and rat and higher than 2g/kg in the mini pig.
- No teratogenic activity
- No adverse effects on fertility
- No carcinogenic activity
- No sex hormones side effects
- No cardiovascular side effects.

This means that: G115, taken as directed, may be used with the utmost safety even for long-term medication.

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We thank the following persons, who have contributed in an essential manner at the execution of all these studies:

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Sandberg: Is there any side effect in man that you could'nt find out in the animal experiments?

Soldati: It was impossible to carry out toxicological studies in man. Anyway, these studies are representative of man and I can tell you that G-115 extract is utilized every year in quantities of 500 million capsules. And we sell this product to over 19 countries. There are many people who take this capsules over a period of 10 years. I think this proves ginseng safe and not toxic.

Shibata: Is the preparation G-115 saponin fraction? Could you tell me what kind of ginseng components are present in G-115 preparation?

Soldati: Since ginsenoside is responsible for the activities of ginseng, we standardized the total ginsenoside content of G-115. The content of ginsenoside is 4.0%. We standardized the extraction method which is published in Planta Medica. But we can't standardize the individual ginsenoside level. That was impossible by this mdthod.

인삼의 독성 연구

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지난 몇해 유럽과 미국의 일부 학자들은 인삼이 장기에 어떤 영향을 미칠 수 있다고 발표하여 저자는 인삼추출물에 대한 안전성을 규명하고자 급, 만성 독성과 심맥계 및 호르몬계에 미치는 영향을 연구하였다.

5년간에 걸쳐 얻어진 이와같은 임상연구 결과는 인삼이 절대적으로 안전하며, 아무런 독성작용과 부작용도 일으키지 않는 것으로 관찰되었다.

REFERENCES

1. Siegel R.K.: Ginseng abuse syndrome, JAMA *241*, 1614 (1979).
2. Palmer B.V., Montgomery A.C.V., and Monteiro J.C.: Ginseng and mastalgia, Brit.

Med. J. 1248 (1978).
3. Punnonen R. and Lukola A.: Oestrogen-like effect of Ginseng, Brit. Med. J. *281,* 1110 (1980).
4. Abramowicz M.: Ginseng, The medical letter (French edition) 2, 25 (1980).
5. Vaille C.: Le ginseng, Le concours medical p. *2267* (1982).
6. Dayer P.: Effects indesirables de quelques therapeutiques non ortodoxes, Pharma-Flash *10,* 4 (1983).
7. Ries C.A. and Sahud M.A.: Agranulocytosis caused by chinese herbal medicines, JAMA *231,* 352 (1975).
8. Liberti L.E. and marderosian A.D.: Evaluation of commercial ginseng products, J. Pharm. Sci. *67,* 1487 (1978).
9. Ruckert K.H.: Quality control of ginseng preparations by means of HPLC, a safe method for the pharmaceutical industry, Proc. of 3rd International Ginseng Symposium, Seoul 1980.
10. Berte F.: Toxicological investigation of the standardized Ginseng Extract G115 after unique administration (LD_{50}), Department of Clinicial Pharmacology. University of Pavia, May 26, 1982.
11. Savel J.: Toxicological investigation of the standardized Ginseng Extract G115, Faculty of Pharmacy, University of Paris, October 27, 1971.
12. Hess F.G., Parent R.A., Cox G.E., Stevens K.R. and Becci P.J.: Reproduction study in rats of Ginseng Extract G115, Food and Chem. Toxicol. *20,* 189 (1982).
13. Hess F.G. Parent R.A., Cox G.E., Stevens K.R. and Becci P.J.: Effect of subchronic feeding of Ginseng Extract G115 in beagle dogs, Food and Chem. Toxicol. *21,* 95 (1983).
14. Trabucchi E.: Teratological investigation of G115, Institute of Pharmacology and Therapeutics, University of Milan, October 8, 1971.
15. Althaus F.R. and Jeenny E.: Effects of Ginseng Extracts and of Ginsennosides at cellular level; DNA repair synthesis in hepatocyte cultures, Institute of Pharmacology and Biochemistry University of Zurich, March 14, 1984.
16. Kyrein H.J.: Steroid Hormons receptors, Deutsche Apoth. Ztg. *116,* 359 (1976).
17. Forgo I., Kayasseh L. and Staub J.J.: Einfluss eines standardisierten Ginseng-Extraktes auf das Allgemeinbefinden, die Reaktionsfahigkit, Lungenfunktion und die gonadalen Hormone, Mediz. Welt *32,* 751 (1981).
18. Buchi K. and Jenny E.: On the interference of the standardized Ginseng Extract G115 and pure Ginsenosides with the cytosolic oestrogen receptor of the mature rat uterus, Hormonlabs., Dept. ginecology, University Hospital Zurich, Nov. 24, 1982.
19. Buchi K. and Jenny E.: On the interference of the standardized Ginseng Extract G115 and pure Ginsenosides with agonists of the progesterone receptor of the human myometriums, Hormonlabs., Dept. ginecology, Unviersity Hospital Zurich, January 18, 1984.
20. Jenny E., Beglinger R., Heider K., Becker M.: On the cardiovascular effects of the standardized Ginseng Extract G115, Institute of Pharmacology and Biochemistry Unviersity of Zurich, September 29, 1982.
21. Paik Y.H.: Effect of Panax ginseng on digitalis action in frog, Chunnam Uidae Chapchi *3,* 119 (1967).