C A M E L Y N

www.camelyn.ge
www.camelyn.com (under preparation)

An independent Georgian Pharmaceutical Company located in Tbilisi, Georgia. "Camelyn LTD"started activities in 2004. "Camelyn" is a privately owned Georgian Scientific-Pharmaceutical Company, established by Ms. Ketevan Maglakelidze, Mzia Maglakelidze and Eliso Khadagishvili (Founders).

The company is dedicated towards identifying and developing immunomodulatory activity, antitumoral, antibacterial and antivirus treatment products, as well as identifying and developing biologically active compounds for use in food industry and cosmetology.

Our branch office in US of America was established in January 2007. "Camelyn US, Inc" takes on the promotion of our products and the representation of our company in South and North America and other countries too. "Camelyn US, Inc" manages our extensive network of promotion agents in the various countries.

Preclinical and Clinical Research

The therapeutic properties of “Camelin M” (initial extract) have been studied by us from 1946. Preclinical, Clinical and Double blind Research with Camelyn's products (Camelyn M1-ampules, Camelyn M2-capsules, Camelyn M3-oitments, Camelyn M4-suppositories) was performed in several Scientific and Clinical Center of Moskow, Leningrad, Tbilisi, Batumi, Quebec, Montreal, Chicago, Bern, Rio de Janeiro, with very promising results.

Antimicrobial Activity of “Camelyn M”

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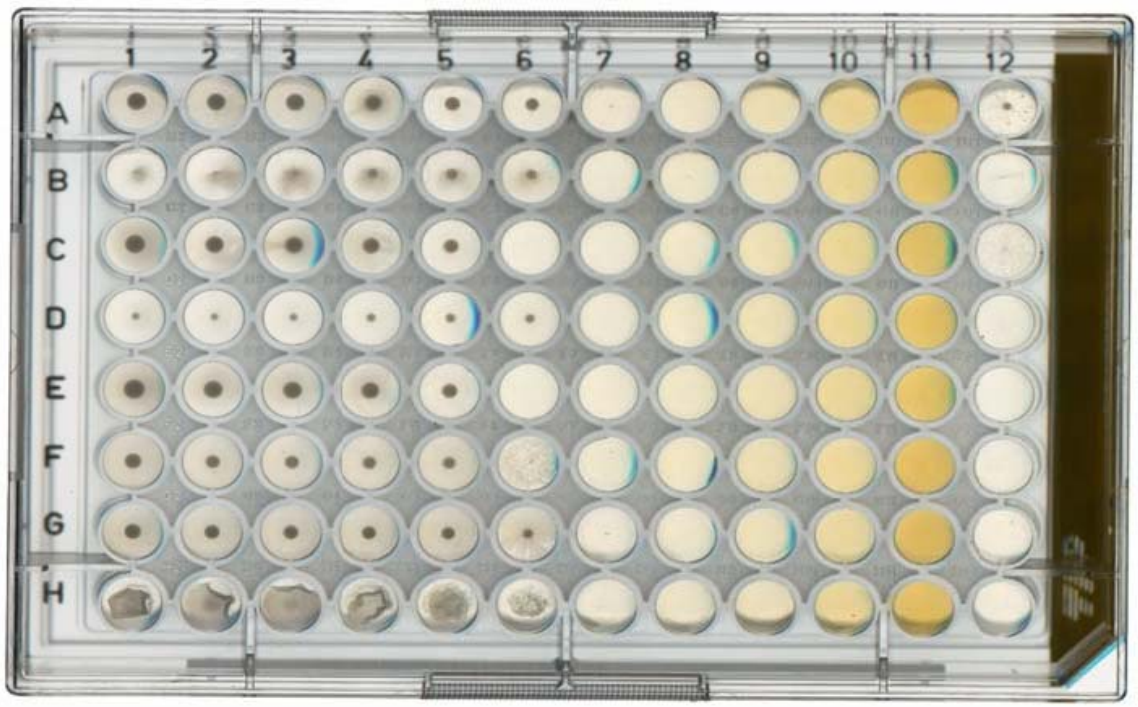

1 2 3 4 5 6 7 8 9 1 0 11 1 2

Fig 1. MICs of “Camelyn M” for **specific antibiotic resistant bacterias** as determined by broth dilution in Müller-Hinton broth .

In vertical-Antibiotic resistant strains of:

A) - *E. faecium* SF11770
B) - *E. faecalis* RE25
C) - *S. aureus* BM3318
D) - *E. faecium* 70/90
E) - *S. aureus* VPKM136
F) - *S. sciuri* CSLA3
G) - *S. haemolyticus* VPS617
H) - *B. cereus* AND934

In horizontal- Concentration of “Camelyn M” %%:

1)- 0.002
2)- 0.004
3)- 0.009
4)- 0.019
5)- 0.039
6)- 0.078
7)- 0.156
8)- 0.310
9)- 0.062
10)- 1.25
11)- 2.50

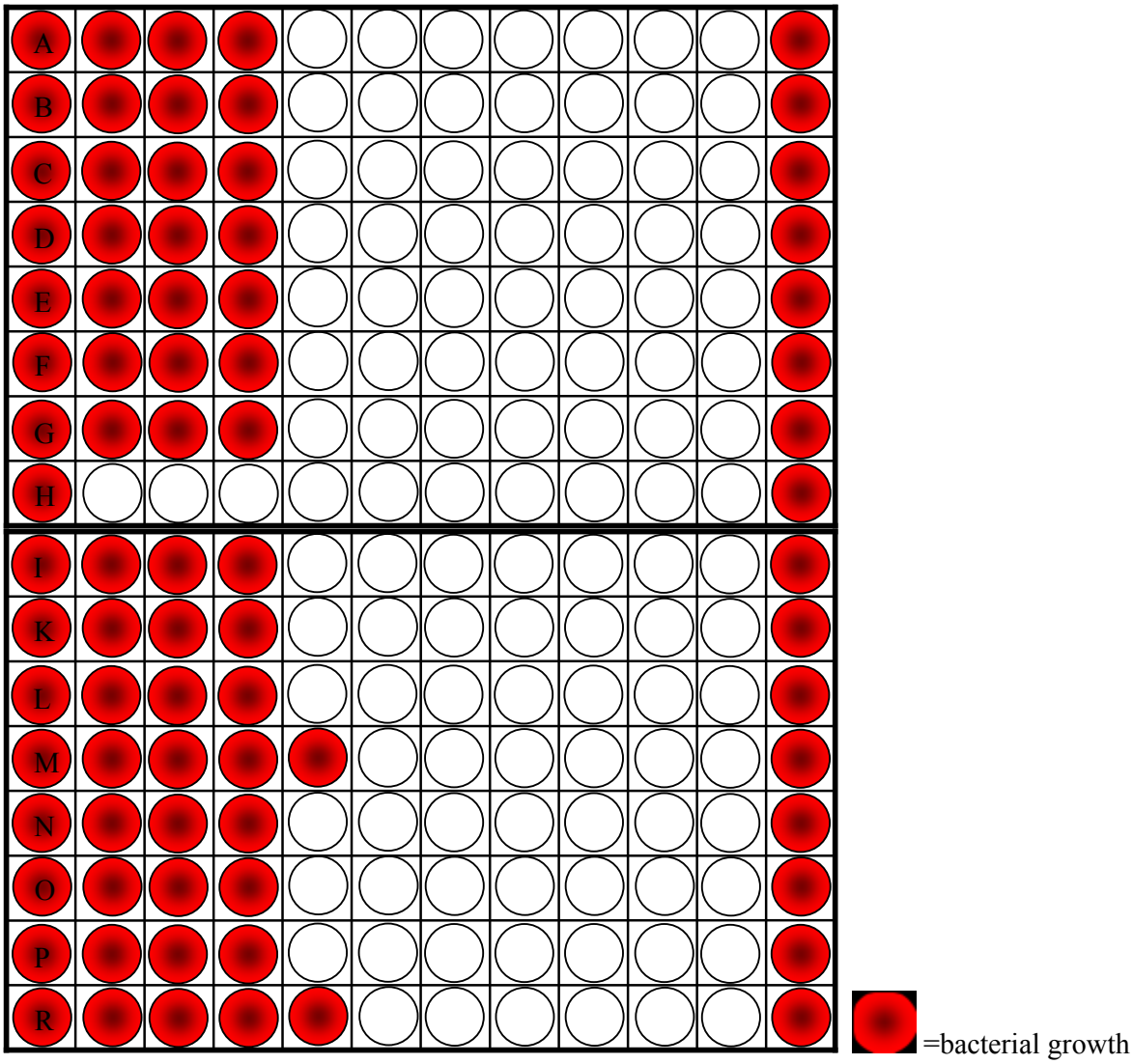

Fig 2. MICs of "Camelyn M" for **specific antibiotic resistant bacterias *of* *Bacilus anthracis and Bacilus cereus***

In vertical-Antibiotic resistant strains of:

A) - ChadA8 ***(B.antracis)***
B) - ChadA1
C) - JF3854
D) - JF3786
E) - JF3788
F) - NCTC8234 (Sterne)
G) - JF3887
H) - JF3888
I) - JF3960
K) - JF3959
L) - JF3961
M) - JF3962 (*B. cereus*)
N) - JF3963
O) - JF3965
P) - JF3966
R) - JF3964

In horizontal- Concentration of "Camelyn M" %%:

1)- 0.002
2)- 0.004
3)- 0.009
4)- 0.019
5)- 0.039
6)- 0.078
7)- 0.156
8)- 0.310
9)- 0.062
10)- 1.25
11)- 2.50

Conclusion

The antimicrobial activities of "Camelyn M" against 8 antibiotic resistant grampositive bacteria strains of A). *E. faecium* SF11770; B).*E. faecalis* RE25; C).*E. faecium* 70/90; D).*S. aureus*

BM3318; E).*S. aureus* VPKM136; F).*S.sciuri* CSLA3; G).*S. haemolyticus* VPS617; H).*B. cereus* AND934, also 11 antibiotic resistant strains of *B.anthracis(ChadA8; ChadA;JF385; JF3786; JF3788; NCTC8234(sterne); JF3887; JF3888* ; *JF3960; JF3959; JF3961* [fig3] and 5 antibiotic resistat strains of *B.cereus: JF3962;* JF3963; JF3964; JF3965; JF3966 were determined. "Camelyn M" at a range of concentration of 0.039-0,31 %- v/v inhibited all bacterial strains.

Antibacterial testing. Antibacterial activity was evaluated using the microdilution method described by Banfi *et al.* (2003) with some modifications. Briefly, exponentially growing bacteria were plated in 96- well round bottom microplates (Costar, Corning Inc.) at a various density. Increasing concentrations of "Camelyn M" compounds in water were then added (100 µL per well).

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A B

Fig 3. MICs of "Camelyn M" for **specific antibiotic resistant bacterias *of* *Bacilus anthracis***
A-after 4 houers inoculation and B-after 4 houers inoculation

Conclusion

The antimicrobial activities of "Camelyn M" against clinical isolates of grampositive bacteria strains of *B.anthracis* were determined. "Camelyn M" at a range of concentration of 1.5-3.0 %- v/v inhibited all bacterial strains.

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Tabele 1. MICs of 1,5; 3,0 and 4,5%% "Camelyn M" for **specific multi and monoresistent bacterias *of* *M.tuberculosis***

Culture	SIRE	Control	1,5%	3,0%	4,5%	Inocul.Date	Result Date
1.$H_{37}Rv$	SSSS	2+	-	-	-	27.04.2007	18.05.2007
2.MDR	RRRR	2+	-	-	-	27.04.2007	18.05.2007
3. Mono-R	RSSS	2+	-	-	-	27.04.2007	18.05.2007
4.Mono-R	SRSS	2+	-	-	-	27.04.2007	18.05.2007
5.MDR	RRRS	2+	-	-	-	27.04.2007	18.05.2007
6. Poly-R	RRSS	2+	-	-	-	27.04.2007	18.05.2007

$H_{37}R_v$-refferenc strain; MDR-multiresistent strain as minimum towards Isoniazid and Rifampin; Poly-R-polyresistent strain towards more than to one preparation; Mono-R-monoresistant strain
S-streptomitsin; R-Rifampin; I-isoniazid; E-ethambutol

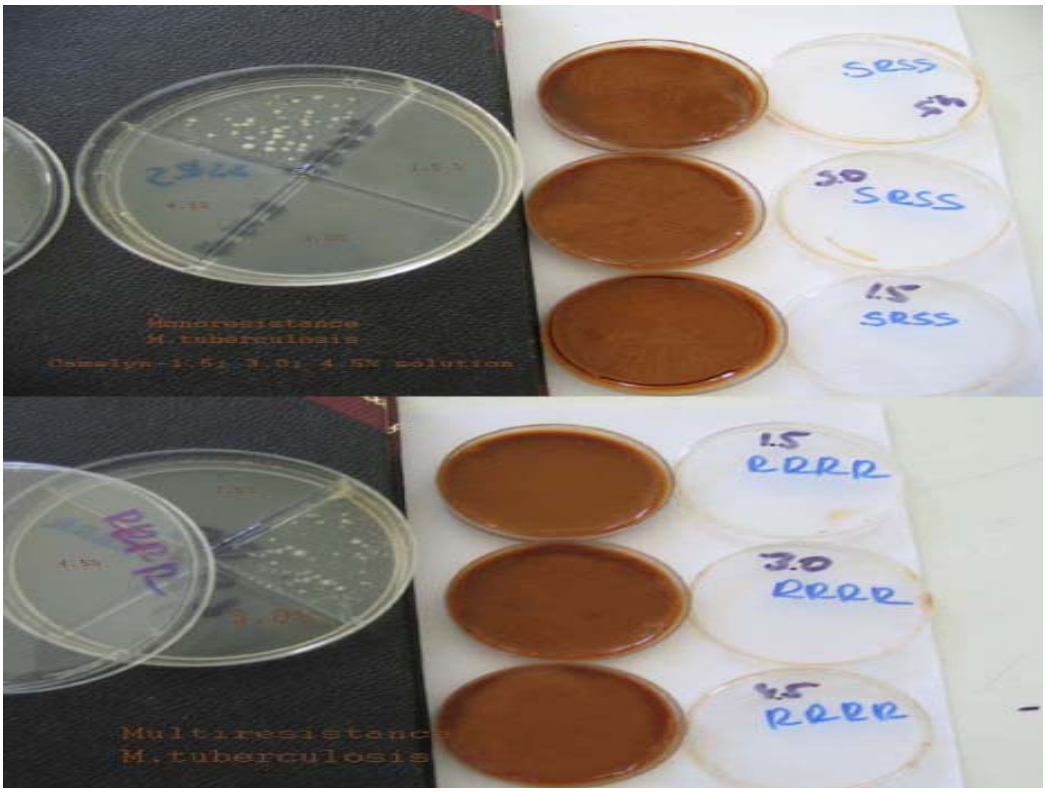

Fig 4. MICs of "Camelyn M" for **specific multi and monoresistent bacterias *of* *M.tuberculosis***

Conclusion

The antimicrobial activities of "Camelyn M" against mono and multiresistent clinical isolate bacteria strains of ***M.tuberculosis*** were determined. "Camelyn M" at a range of concentration of 1.5-3.0 %- v/v inhibited all bacterial strains.

Antiviral Activity of "Camelyn M"

4). Montreal

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Anti-viral Activity in CBMC's (Cord Blood Mononuclear Cells)

Method: The CBMC's were plated in wells, treated with serial dilutions of the preparation for 2 hours, and then infected with HIV-1 (IIIB, Wildtype, Laboratory Strain). After 6 days in culture, the RT activity was measured. Cell counts were determined using a duplicate plate with a mock infection.

Results

Dilution	RT Activity (CPM)		Cell Number (x 10_6)
0	107192	172047	4.3
1: 2000000000	158403	188325	4.4
1: 200000000	176987	176020	4.0
1: 20000000	166766	201221	3.9
1: 2000000	104831	103171	3.3
1: 200000	18785	47706	1.8
1: 20000	3838	3223	1.2
1: 2000	1886	2755	1.3
1: 200	3427	3518	Not done
1: 20	2611	3831	Not done

Conclusions: The preparation showed significant anti-viral effect. The decrease in RT activity corresponds to a decrease in cell number. (in red). 3TC was run as a control compound, and gave an IC_{50} value of 0.017 µM, as expected.

This compound exhibited potent inhibitory effect on the replication of HIV-1 laboratory strains (IC50s 0.017 µM /mL) and HIV-1 clinical isolate strain KK-1(0.012 µM /mL). Camelyn inhibit virus adsorption, giant cell formation, reverse transcriptase activity and integration of the HIV genome into the chromosomal DNA.

Antifungal Activity of "Camelyn M"

5). Quebec

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Candida species are an important cause of opportunistic infection in the oral cavity of immunocompromised patients, especially HIV infected patients. The activities of "Camelyn M", isolated from special sort of honey, distributed in one of region of Georgia, were evaluated *in vitro*. Camelyn M exhibited potent in vitro activities against Fluconazole**(FLC)**-resistant strains of *Candida albicans* , *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*, with MICs at which 90% of isolates were inhibited of 0.012 µg/ml, respectively.

TABLE 1. Antifungal spectrum of "Camelyn M"

Organism and strain	MIC (µg/ml)			
	Camelyn M	FLC	ITC	AMB
Candida albicans ATCC 24433	0.012	0.25	0.016	0.12
Candida glabrata ATCC 90030	0.012	4.00	0.12	0.12
Candida tropicalis ATCC 750	0.012	2.00	0.06	0.25
Candida parapsilosis ATCC 22019	0.012	2.00	0.03	0.5
Candida krusei TIMM3378	0.012	32.00	0.06	0.25
Candida guilliermondii ATCC 9390	0.012	2.00	0.03	0.06

TABLE 2. In vitro antifungal activity of "Camelyn M" against clinical isolate of *Candida albicans.*

Organism (clinical isolates) and agent	MIC (µg/ml)	
	50%	90%
Candida albicans		
"Camelyn M"	0.012	0.012
FLC	0.25-8	1.00
ITC	0.016	0.03
AMB	0.12	0.12

Table 1 shows the spectrum of activities of "Camelyn M" and other reference against various fungal strains. "Camelyn M" exhibited potent activities against *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. guilliermondii*, and *C. neoformans*, with MICs ranging from 0.012 µg/ml for all the above listed cases. Table 2 shows the MICs of "Camelyn M" and other reference agents for clinical yeast isolate of *C. albicans*. Table 2 shows the results for *C. albicans* separately for FLC-susceptible (FLC-S) strains (FLC MICs, ≤8 µg/ml) and FLC-susceptible dose-dependent (FLC-S-DD) and FLC-resistant (FLC-R) strains (FLC MICs, ≥16 µg/ml), according to the guidelines of NCCLS document M27-A2. "Camelyn M" exhibited potent activities against *C. albicans* (FLC-S), with MICs at which 90% of isolate are inhibited (MIC_{90}s) of 0.012 µg/ml. "Camelin M" also exhibited potent activities against the FLC-S-DD and FLC-R strains of *C. albicans* (MIC range, 0.012 µg/ml).

Conclusion

In conclusion, the results of the present study suggest that "Camelyn M" is a promising compound, in particular, for the treatment of disseminated or mucosal infections induced by *C. albicans*, including FLC-resistant strains.

Anticancer Activity of "Camelyn M"

6) Tbilisi; Quebec.

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Summary: The effects of therapy with "Camelyn M", active compound obtained from special sort of honey in one of region of Georgia, were examined in 15500 cancer patients, between 1946 and 1968. Experiments we are carried out, at the Tbilisi State Medical Institute, Leningrad Institute of Oncology of the Academy of Sciences of the USSR and at the Moscow Institute of the Experimental Pathology and Therapy of Tumor, on the III and IV stage of disease, mainly on the terminal stage of deeply lying tumors of internal organs. Patients were treated with "Camelin M" after conventional therapies such as surgery, chemotherapy and radiation. All patients had a pathological report confirming a diagnosis indicating the type and stage of cancer. The type of chemotherapy was obtained from medical records. Significant anticancer activity by "Camelin M" was observed in patients given intramuscularly 5 g of "Camelin M" 3 times per day with Novocain. Number of patients with wearies cancer decease were as follows: 1.Carcinoma of prostate gland-2000; 2.Carcinoma of uterine cervix-1200; 3.Ovarian carcinoma-1800; 4.Carcinoma of urinary bladder-2000; 5.Renal tumor-1000; 6.Colon cancer-3000; 7. Fibrosarcoma-500; 8.Carcinoma of stomach-1500, 9. Lung cancer-500; 10.Oesophagus cancer-1000; 11.Larynx tumor-500; 12. Endothelioma-500. "Camelyn M" displays a strong inhibiting action on the growth of certain forms of tumors in experiment, as well as in clinic, to the point of their full regression. Two mechanisms by which "Camelyn" exerts its effect were investigated. The first was imunomodulation. The mechanism of action involves the stimulation of three important cytokines: IL-2, IFN-γ, and TNF-α. The second was direct anticancer properties. *In vitro* experiments, "Camelin M" was found to be active against DLD-1 colon carcinoma and A-549 lung carcinoma cells, with IC50 values of 0.0063 ± 0.0006 μg/mL and 0.0078 ± 0.0005 μg/mL , respectively.I*n vivo* studies in mice showed that "Camelyn M" possesses suppressive effects on tumour cell growth, 0.1 ml 35% solution of "Camelyn M" caused full regression of tumor in maic. It is concluded that the high anticancer effect of "Camelyn M" and the absence of notable side-effects make "Camelyn" a promising therapeutic agent for the treatment of cancer patients. At the same time, the preparation stimulates growth and reproduction of young connective tissues.

The preparation Camelyn was obtained in the liquid form from the special sort of honey .
PH=3-4 dilution 1:4, 1:8 does not change pH.

Method: Tests in mice were carried out using of 0,1 ml dose of 35% solution of the preparation "Camelyn M" on 100,0 g weight of the animal . The preparation was introduced intraperitoneally every day during two weeks. Tumours were measured every 4th day, calculation of average diameter of tumours was performed by the following formula:

$$D = \frac{a+b+c}{3}$$

Where:
D – average diameter of the tumour,
a - length of the tumour,
b – width of the tumour,
c - height of the tumour.

Growth inhibition of the tumour was calculated by the following formula:

$$t = \frac{MB - MO}{MB} \times 100$$

Where:
MB - is an average weight of the tumour in control,
MO – is an average weight of the tumour during tests.

***In vitro* assay**

Cell culture

The human lung carcinoma A-549 (ATCC #CCL-185), colon adenocarcinoma DLD-1 (ATCC #CCL-221), and murine macrophage RAW 264.7 (ATCC #TIB-71) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, USA). The A-549, DLD-1 cell lines were grown in

Minimum Essential Medium with Earle's salts, while the RAW 264.7 cell line was grown in Dulbecco's modified Eagle's medium (Mediatech Cellgro®, Herndon, USA). Both media were supplemented with 10% fetal calf serum (Hyclone, Logan, USA), solution of vitamins (1X), sodium pyruvate (1X), nonessential amino acids (1X), penicillin (100 IU) and streptomycin (100µg/ml) (Mediatech Cellgro®). Cells were cultured in a humidified atmosphere at 37 ºC in 5% CO2.

*Cytotoxicity assay:*Exponentially growing cells were plated at a density of 5 x 103 cells per well in 96-well microplates (Costar, Corning inc.) in 100 µl of culture medium and were allowed to adhere for 16 hours before treatment. Then, 100 µl of increasing concentrations of extract or pure compounds dissolved in the appropriate solvent (Sigma-Aldrich) were added. The final concentration of solvent in the culture medium was maintained at 0.5% (volume/volume) to avoid solvent toxicity. The cells were incubated for 48 h in the absence or in the presence of extract. Cytotoxicity was assessed using the resazurin reduction test as described by O'Brien (O'Brien et al., 2000). Fluorescence was measured on an automated 96-well Fluoroskan Ascent Fl™ plate reader (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxicity was expressed as the concentration of extract or compound inhibiting cell growth by 50% (IC50).

***In vivo* assay**

*Tumour cell-lines:*Mouse M-1 sarcoma line cells was passaged *in vivo* in BALB/c mice. Twenty-one days after inoculation, the tumors were excised, gently teased over stainless steel screens, washed in RPMI 1640 medium and resuspended in PBS (1x106 viable cells/ml).

Material

The solvents were purchased from EMD (Canada). Fetal calf serum and RPMI 1640 medium were purchased from Gibco BRL (Rockville, MD), HEPES, Novocaine, Concanavalin A, All other reagents were purchased from Sigma–Aldrich.

Cytokines: For evaluation of cytokine production, we incubated purified spleen cells from each animal (2x106 cells/ml in RPMI 1640 medium with 5% FCS) in wells of a 24-well tissue culture plate. After addition of 1 µg of Concanavalin A or 10 µg of LPS per well, cells were incubated for 72 hr in a humidified incubator (37°C, 5% CO2). At the endpoint of incubation, supernatants were collected, filtered through 0.45 µm filter and tested for the presence of IL-2, IFN-γ and TNF-α. Levels of cytokines were measured by commercial ELISA in accordance with the protocol for Cytoscreen™ of BioSource International, Inc (Camarillo, CA).

Treatment protocol- In patients, the preparation "Camelyn M" was injected intramuscularly in rising doses from 1 to 5 g 3 times per day with Novocaine. At first 2-3 g 0,5-1% solution of Novocain was administered, accuse is left during 2-3 minutes, after the preparation was added reaching 5 g, duration of treatment 5-6 days, further gradually is administered 2-3 g.

Results

*Evaluation of cytotoxicity against tumor cell lines:*The anticancer activity of both 35%; 100% solution and powder (dried) form of preparation of "Camelin M" was evaluated against colon carcinoma cell line DLD-1 and lung carcinoma cell line A-549. As shown in Table 3. the powder form of "Camelin M" was inactive against DLD-1 and A-549 with an IC50 value higher than 200 µg/ml. In contrast, the results presented in table 1. show that the 35% and 100% solution of "Camelin M" was active against A-549 and DLD-1, with IC50 value of 0.0063 ± 0.0006 µg/ml; 0.0078 ± 0.0005 µg/ml and 0.0070 ± 0.0004 µg/ml; 0.0104 ± 0.0002 µg/ml, respectively.

Table 3. Cytotoxic effect of 35% ;100% solution and powder (dried) form of preparation of "Camelin M" against carcinoma colon cell lines DLD-1 and lung adenocarcinoma cell lines (A-549).

Cancer cells	A-549	DLD-1
Camelin-Powder	>200 µg/mL	>200 µg/mL
Camelin -M1 35%	0.0063 ± 0.0006 µg/mL	0.0078 ± 0.0005 µg/mL
Camelin-SBS-100%	0.0070 ± 0.0004 µg/mL	0.0104 ± 0.0002 µg/mL

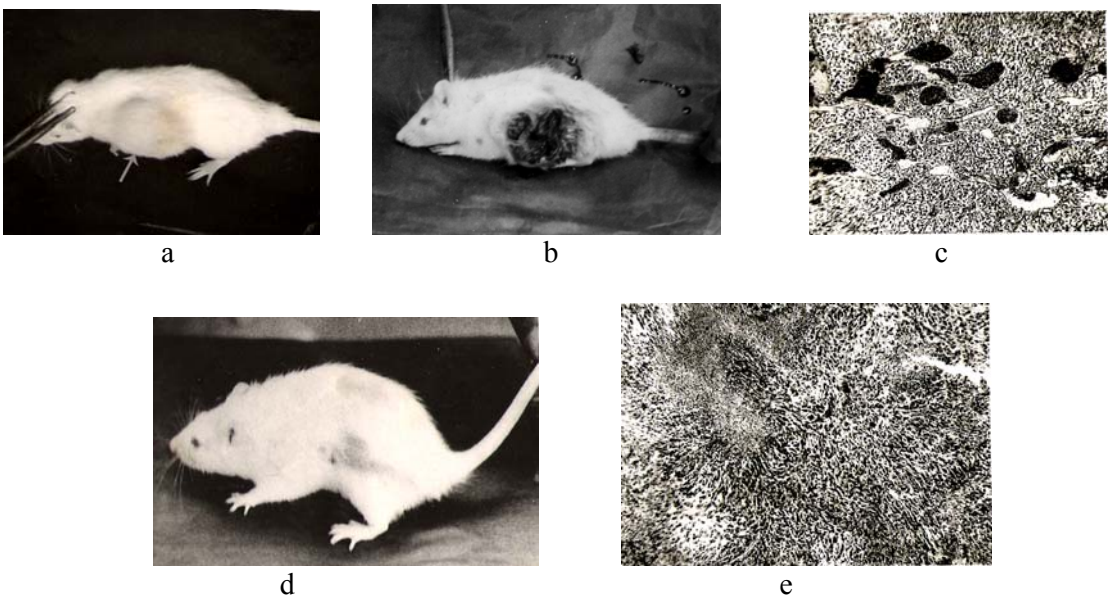
As it follows from the Table 4. establishment of tumors after their inoculation in mice in all cases accounts for about 80-90%.

Table 4.

Peculiarities of the Sarcoma M-1 growth Transplanted After inoculation of tumor and treatment with "Cameyin M" in mice

Group	Inoculation of tumor on the 10th day				Treatment with "Camelyn M"					
					After 20 days	After 1,5 month				
	Number of animals	Was inoculated	Wasn't inoculated	%	Size of tumor (mm)	Disappeared	Remained	%	Size of tumor (mm)	Died due to tumor
I	10	8	2	80	5,7	7	1	87.5	0,2	
II	10	9	1	90	3,4	9	0	100	0	
III	10	8	2	80	7	8	0	100	0	
IV	9	5	4	55,5	6,6	4	0	100	0,4	
V	10	8	2	80	6,4	8	0	100	0	
Control	25	20	5	80	17,8	-	-	-	-	20 rats

As it follows from the Table 4, inoculation of tumors (Fig: 5-a,b and c) in the control, as well as in the test groups, is about the same, though in this case the size of tumors after 20 days of treatmen twith "Camelin M" in test groups is significantly less, than in the control (Table 4.). After 1,5 months considerable part of tumors in testanimals resorpted (Table 4; Figure 5-d and e) , when in the control group the tumors continued to grow and all the animals died.



a b c d e

Fig 5. Rat sarcoma before and after treatment with preparation of "Camelin M"
a,b-infected mice from control group; c- histomorphological picture of tumor in control mice.
d-mice from experimental group after1,5 months (tumor resorpted); e- histomorphological picture of tumor in experimental mice (tumor resorpted).

Investigation of the effect of oral introduction of the preparation on the tumor growth was interesting. For clarification of the issue we performed the following tests: 30 rats were tested: 10 of which constituted the 1st group, 10-II and 10 – the control. To the rats of the first group during 10 days one time a day was given 0,5 ml of the 10% solution of "Camelyn M", after which sarcoma M-1 was inoculated to them. (Table 5). To the rats of group II in the same way during 10 days was given the preparation in the same doze, following to which sarcoma M-1 was inoculated to them. After inoculation of the tumor the animals continued to receive "Camelyn M" in the same doze one time a day during 40 days (Table 5).

Consequently, in the organism preliminarily prepared with "Camelyn M", the tumor cells loose considerably their malignant properties and the tumors resorpt. This is clearly seen, if introduction of the preparation is continued during a long time after inoculation. These observations brought us to the following the working assumption of the mechanism of "Camelyn M" action. Under the effect of "Camelyn M"malignancy of the tumor cells is reduced, at that we could think that this effect depends on some changes in the antigen composition of tumors leading to the changes of immunobiological reactivity of the organism. Consequently, the tumors processed with "Camelyn M" regress, that, in its part, causes the immunobiological shifts in the organism, creating the condition of resistance to the given tumor strain.

Table 5.

Peculiarities of the Sarcoma M-1 growth during oral introduction of "Camelin M" in mice

Group	Duration of Introduction of the Preparation, days	Inoculation of tumor on the 10th day					Resorption after 1,5 month				
		Number of animals	Was inoculated	Wasn't inoculated	%	Size of tumor, mm	Disappeared	Remained	%	Size of tumor, mm	Died due to tumor
I II Control	10 40 -	10 10 10	5 3 8	5 7 2	50 30 80	6 3 17, 3	3 2 -	2 1 8	60 66,6 -	3,6 0,6 28,3	 8 rats

Evidence of orally-administered "Camelin M" immunomodulatory activity was also demonstrated through effects on the production of three different cytokines, IL-2, IFN-γ, and TNF-ά (Table 6). The production of these three cytokines was measured after a 72 hr *in vitro* incubation of spleen cells isolated from control and "Camelin M" administered animals. For all three tested cytokines, oral administration of "Camelin M" resulted in significantly-increased cytokine levels IL-2 (3.1-fold), IFN-γ. (4.4-fold), and TNF-ά (8.7-fold), P< 0.05) over control animals.

Table 6. *in vivo* Effects of oral administration of Camelin on cytokines

Cytokine	Control	"Camelin M"
IL-2	14.5+/- 0.7 pg/ml	45.6 +/- 4.1 pg/ml*
IFN-γ	163.4 +/-12. 4 pg/ml	721.3 +/- 64.4 pg/ml*
TNF-(α)	362.2 +/- 43.2 pg/ml	3156.2 +/- 129.9 pg/ml*

*P values were determined using a student's T-test (P<0.05 for IL-2, IFN-γ, and TNF-α).

Clinical observations were examined in 15500 cancer patients. Many of cases were demonstrated on the united session of surgical, oncologic and urological societies of Georgia on February 7, 1968; March 16, 1975; April 1998 and Julay 3, 2003.

During analysis of the carried out observations first of all becomes clear that at present we have all the reasons for optimistic evaluation of antitumor properties of the preparation. "Camelin M" displays a strong inhibiting action on the growth of certain forms of tumors in experiment, as well as in clinic, to the point of their full regression. It should be noted, that all the patients, with the exception of some – with larynx tumor, had far gone forms of disease, whereas some were operated many times in the past, one of them – 6 times. In spite of the grave condition of patients, terminal stage of the disease, treatment with

"Camelin M" appeared to be efficient. The patients were observed during the period beginning from a year to 10 years after treatment.

Among 15500 patients under observation relapse was marked among 40. For example: at carcinoma of the urinary bladder (case histories N 7743,) in 4 years after treatment the patient was hospitalized with a relapse, after which he died. The second (case histories N 7743,) – in 7 years after treatment went to the IInd urological department of the Central Republican Hospital in October – after the operation was discharged in good condition. The third (case histories N 7743,), suffering from Carcinoma of stomach , in 2 years after treatment had a relapse. At present he is being treated with "Camelin M". The forth (case histories N 7743,) patient suffering from carcinoma of the prostate gland with metastasis of ischial bone and urine retention, following to the 5 year of treatment went to the 1st urological department of the Central Republican Hospital. The operation cystostomia was carried out, at opening of the urinary bladder a large size stone was found. The patient was discharged from the clinic in good condition.

Sometimes large tumors are resolved fully that, of course, improves general condition of the patient. In separate cases, carcinoma resolve only partially, in others – fully, even in the existence of larger tumors. Such cases are not many, but they are still marked. At Ovarian carcinoma, for example, when the patient was operated 6 times, after each operation the tumor was relapsed in one and a half, two months. After the 6th operation the patient began administering of the preparation "Camelin M". During the first course of treatment the tumor was resolved. Four years passed, the patient is healthy. There are no signs of relapse. The other patient (case history N 1285) suffering from sarcoma of parietal lobe with the defect of skull bone (Fig 6.), was operated four times. After each operation the tumor grew again. During the first course of treatment with preparatin of "Camelin M", defect of the skull bone was restored. At present the patient is healthy; after the treatment 36 years have passed.

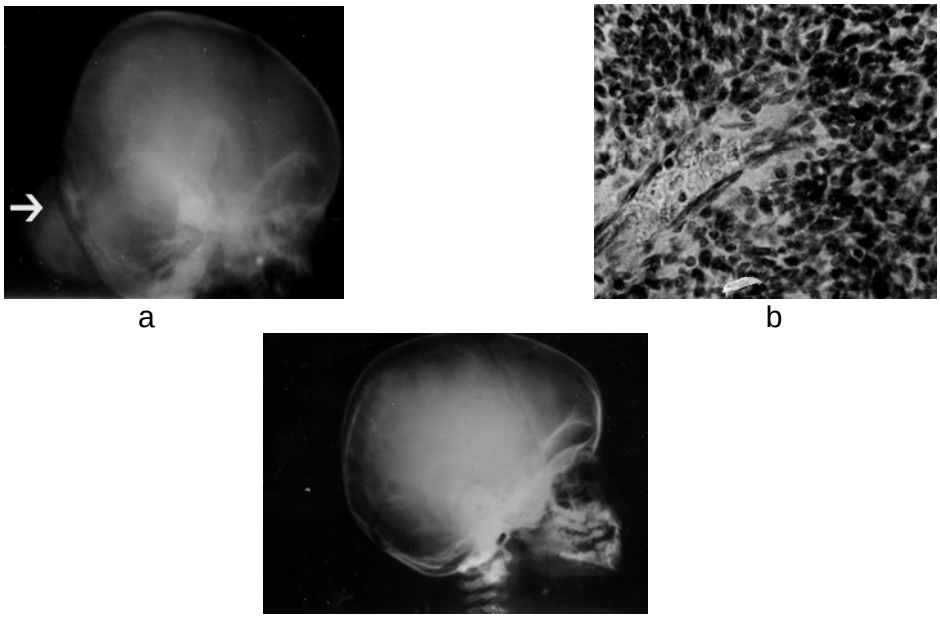
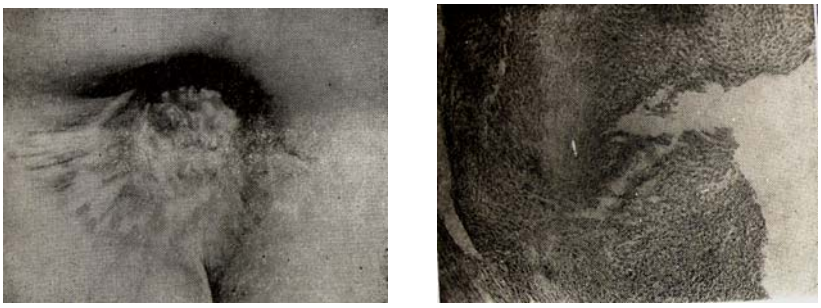
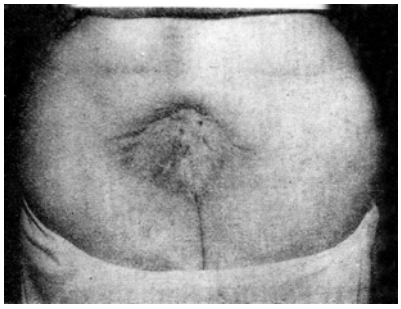


Fig.6. Defect of the skull before and after treatment of "Camelyn M" .





c

Fig. 7. Sarcoma of sacral part before and after treatment of "Camelyn M".
a- before treatment of "Camelyn M"; b- histomorphological analysis of the tumor
c- The same case after the third course of treatment of "Camelyn M".

On the basis analysis of result of own observations, the following conclusions may be made:
1. Apparently, the preparation "Camelyn M" has an antitumoral properties and is a strong biological stimulator of the human organism.
2. The preparation is not toxic, has no side effects. In the cases of recovery of neoplasm, the relapse is not developed, especially in the existence of sarcoma.
3. Antitumoral action of the preparation is revealed in the cases of initial cancers. The preparation must be applied in tablets for the prevention purposes, especially against sarcoma.
4. On the terminal stage of cancer in the majority of cases, the preparations gives improvement with prolongation of the patient's life, in isolated instances the full recovery.

Toxicity of "Camelyn M"

Lethal and toxic doses of the preparation Camelyn were determined by means of white mice, guinea-pigs and dogs.

It was found that the white mice weighing 30-39 g assimilate well the subcutaneous injection of 0,5 ml 20% solution of the preparation Camelyn. Observations performed on mice during a month proved that this dose is not toxic. The mice gain weight, reproduce, do not change their behavior. In composition of the blood of the part of animals insignificant increase of the leucocytes on the expense of neutrophils, but it does not exceed the scopes of norm. Autopsy of the animals after 30 days of observation no pathological changes in the internal organs were not marked.

20% of solution of the preparation was injected subcutaneously in the quantity of 9 ml the weight of animals (guinea-pigs) being 275 g, in the quantity 11 ml – the weight of animals being 300 g, etc. The dose appeared to be lethal. All the animals died on the second day after injecting of the solution. By autopsy and histomorphological studies of the organs was determined that this dose causes dystrophic occurrences in the parenchymatous organs of the guinea-pigs. Sharp dystrophic occurrences were marked in kidneys, as well as cyanosis and stasis; in adrenal glands – hemorrhage, in the heart muscle – edema.

During the following series of tests the preparation dose was reduced twice: to the guinea-pig the weight being 300 g 3-4 ml of preparation was injected. 345 g – 6 ml, etc. Part of the animals died, the other survived and gained weight. During the last series of tests 2 ml of the preparation was injected. It appeared that subcutaneous injection of 20% of the preparation Camelyn in the quantity 2 ml the guinea-pigs withstand well, even injected every day during 20-25 days. During life, as well as after autopsy of animals no pathological changes were marked.

To the dogs during the first series of tests the preparation Camelyn was injected in a large dose. For example, the dog weighing 43,5kg -165 ml 20% solution. In spite of such a large dose, they withstood the preparation, felt well, did not change behavior, preserved playfulness and appetite. During the second series of tests on dogs we decided to test the effect of comparatively small doses of the preparation. The

dog weighing 13 kg was injected intravenously 65 ml of 20% preparation; the dog weighing 14 kg – 46 ml, and the dog weighing 16,5 kg – 55 ml. Not only the behavior of the animals was observed, but the blood pressure, respiration, medullogram and hemogram. With the purpose of studying the effect of the preparation on the blood pressure and respiration, the tests were performed on 9 dogs.

The dog No 1, weighing 13 kg, 20% solution of the Camelyn was injected intravenously, in the quantity 170 ml during 2 minutes and 30 seconds, accordingly, about 13 ml on 1 kg of the animal weight. The blood pressure during the first seconds of the preparation injection dropped from 84 ml of mercury column to 45 ml. During the following seconds it began to improve and at the end of injection it became 90-96 ml, after 20 minutes after injection it returned to the initial value. Control of the dog was carried out during a month. During the term no pathological changes were marked.

The dog N 2, weighing 16 kg, 495 ml of 20% solution of the preparation was injected intravenously during 40 minutes and 38 seconds. Before injection of the preparation it increased to 120 mm, and then reduced to 110 mm. During the one month control no pathological changes were marked in the dog.

The dog N 3, weighing 13 kg, 65 ml of the preparation was injected intravenously during 3 minutes and 22 seconds. It makes about 5 ml of 20% preparation per 1 kg of the animal. The blood pressure of the given dog before injection was 60 mm, during the first minutes after injecting it dropped to 40 mm, and after 10 minutes returned to the initial index and was preserved to the end of the test. The control was performed during a month.

The dog N 4, weighing 14 kg, the preparation was injected intravenously in the quantity of 46 ml during 4 minutes and 30 seconds, accordingly, 3 ml of 2% solution of the preparation Camelyn per 1 kg of the animal weight. The blood pressure during the preparation injection was preserved on the initial level (110 mm). Immediately after its injection it dropped to 100 mm, and after 40 minutes after the injection to 30 mm, and after 50 minutes returned to the initial value. No changes were observed in respiration. During a month control no pathological changes were marked in the dog .

The dog N 5, weighing 15,5 kg, 50 ml of 20% solution of the preparation Camelyn was injected intravenously during 5 minutes, about 3 ml per 1 kg of the animal weight. Before the preparation injection the blood pressure increased to 99 mm, and in 10 minutes returned to the initial level and was preserved during the whole test. The control was performed during a month. The pathological changes were not observed.

Thus, the preparation Camelyn injected to the animals in the definite dose does not cause any toxic events.

Antileishmanial Activity of “Camelyn M”

7) Brazil

Dr. Ricardo Ramos Mendonça Filho

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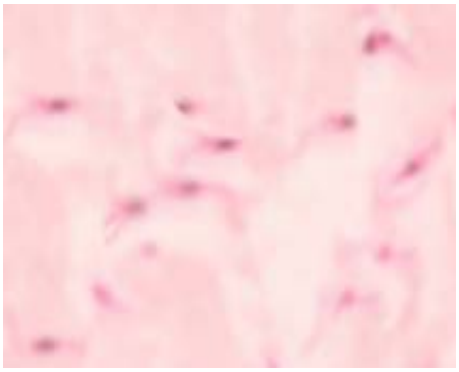
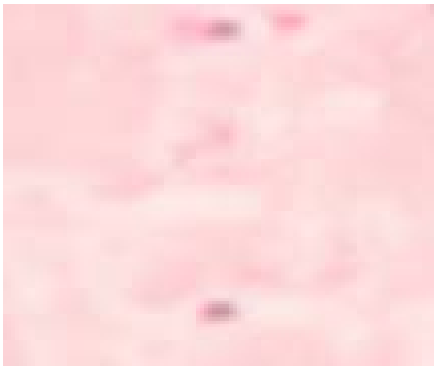
Instituto de Microbiologia Professor Paulo de Góes – IMPPG; Departamento de Microbiologia Geral, sala 050 ; Laboratório de Estruturas de Superfície de Microrganismos ;Avenida Carlos Chagas Filho, 373, Cidade Universitária,RJ 21941-902, Brasil.

Diseases caused by protozoans are responsible for considerable morbidity and mortality throughout the world, but predominantly in the tropics and subtropics. Present treatment regimens for these diseases have severe limitations, and new drugs are urgently required. In this regard, natural products have made and are continuing to make important contributions to this area of therapeutics. At present, leishmaniasis threatens 350 million people worldwide, and an estimated 1.5 million to 2 million new cases occur annually.
Experiment for evaluation of the cytotoxic effect was the red blood cell lysis assay. Briefly, a 4% suspension of freshly defibrinized sheep blood was prepared in sterile 5% glucose solution. Different concentrations of isolated compound were added to each test tube. The red blood cell suspension was then added, the contents were gently mixed, and the tubes were incubated at 37°C. The minimum lytic concentration is defined as the lowest concentration of a test compound that produces complete or partial lysis of erythrocytes
Parasites. Strain of *L. amazonensis*, originally isolated from a human case of diffuse cutaneous leishmaniasis by C. A. Cuba-Cuba (University of Brasília, Brasília, Distrito Federal, Brazil) was used in the present study. It was maintained at 28°C in Warren's medium (brain heart infusion plus hemin and folic acid) supplemented with 10% heat-inactivated fetal bovine serum in a tissue flask.
Antileishmanial activity. Promastigote forms of *L. amazonensis* (10^6 parasites) were grown in a 24-well plate in Warren's medium supplemented with 10% inactivated fetal bovine serum and different concentrations of “Camelyn M”. The cell density for each treatment was determined daily in a hemocytometer (Improved Double Neubauer) with an optical microscope. Each experiment was performed twice on different occasions.





Light microscopy image

Fig 1. The test tubes with parasites (*L. amazonensis),* bifore treatment with “Camelyn M”

Fig 2. The same case after 30 min of treatment with 3% solution of “Camelyn M”. Twisting and lysis of parasites is visible.

Here we report for the first time a novel pharmacological activity in the “Camelyn M”, which exhibited potent activity against *L. amazonensis*. “Camelyn M” inhibited promastigote growth with an IC_{50} of 0,3 µg/ml, leading us to carry out a bioassay-guided fractionation of the antileishmanial activity. The liquid fraction of “Camelyn M” showed a greater inhibitory effect than the powder with the IC_{50} of 36 µg/ml.

Antibacterial and Antifungal Activity of “Camelyn M”

Brazil

Dr. Ricardo Ramos Mendonça Filho

TABLE 1.
MICs of “Camelyn M” for the pathogenic strains of **antibiotic resistance** micro organisms

Strains	**MIC (µg/ml) of:**			
Gram(+) bacteria	Causes types of infection	**Camelyn M**	**Penicillin**	**Flucozanol**
Streptococcus pneumoniae	pneumonia ; acute sinusitis, otitis media, meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis	0.0030	0.03	0.015
Streptococcus mutans	tooth decay	0.0025	0.25	0.03
Streptococcus pyogenes	pharyngitis; impetigo; impetigo; necrotizing fasciitis	0.0032	0.125	0.125
Enterococcus faecalis	endocarditis, bladder, prostate, epididymal	0.023	8.0	0.5
Staphylococcus epidermidis	is an important cause of infection in patients whose immune system is compromised.	0.005	2.0	1.0
Listeria monocytogenes	listeriosis; septicemia,meningitis ; meningoencephalitis, encephalitis	0.003	3.0	1.0
Clostridium perfringens	tissue necrosis, bacteremia, emphysematous cholecystitis, gas gangrene myonecrosis.	0.003	1.0	1.0
Nocardia brasiliensis	nocardiosis encephalitis cerebral abscess	0.016	ND	1.0
Gram (-) bacteria				
Shigella dysenteriae	shigellosis (bacillary	0.023	2.0	2.0

Strains	MIC (µg/ml) of:			
Gram(+) bacteria	Causes types of infection	**Camelyn M**	**Penicillin**	**Flucozanol**
	dysentery).			
Pseudomonas aeruginosa	typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections	0.018	4.0	2.0
Fungy			8.0	2.0
Trichophyton rubrum	athlete's foot , jock itch and ringworm	0.012	8.0	3.0
Trichophyton mentagrophytes	dermatoinfections	0.034	ND	4.0
Fonsecaea pedrosoi	Cerebral phacohyphomycosis ("chromoblastomycosis")	0.023	4.0	4.0
Cryptococcus neoformans	cryptococcosis.	0.002	8.0	4.0
Candia albicans	candidoses	0.002	8.0	4.0

ND=not determined

Conclusuin

In summary, “Camelyn M” exhibited potent bactericidal activity against pathogenic fungy and bacterias in the murine thigh infection model. The relationship between *T*>MIC and bactericidal activity can be characterized with the sigmoid E_{max} model.

Scientific- Practical Centre of thermal damage
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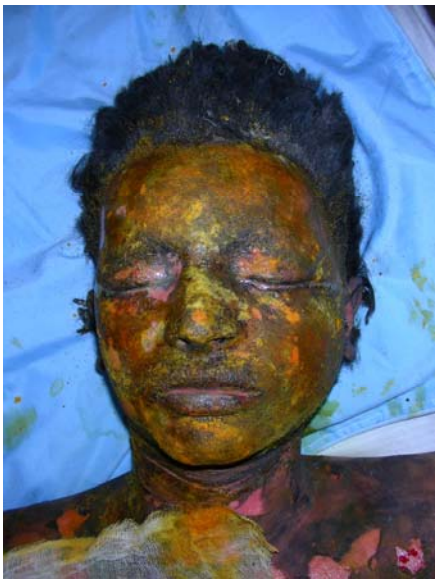
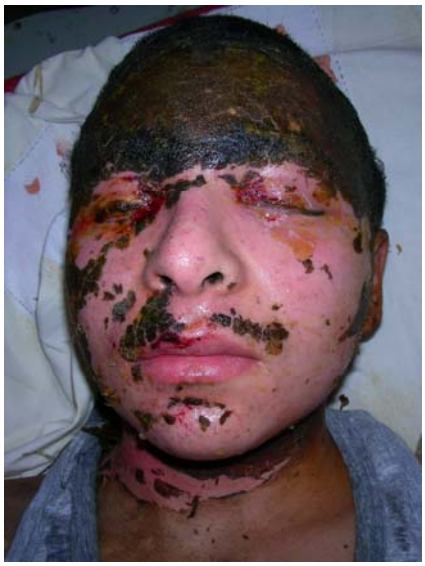
Tbilisi, Georgia

Thermal injury and burn disease

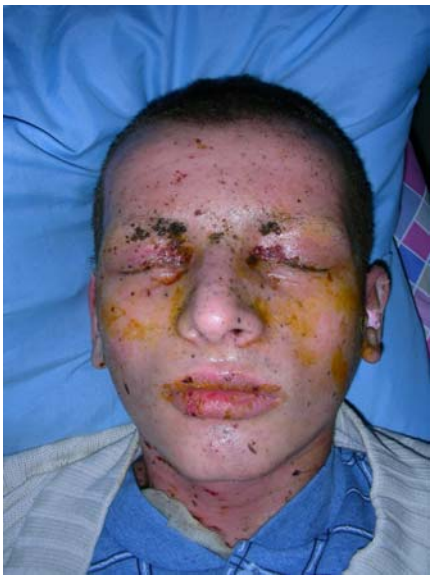


a



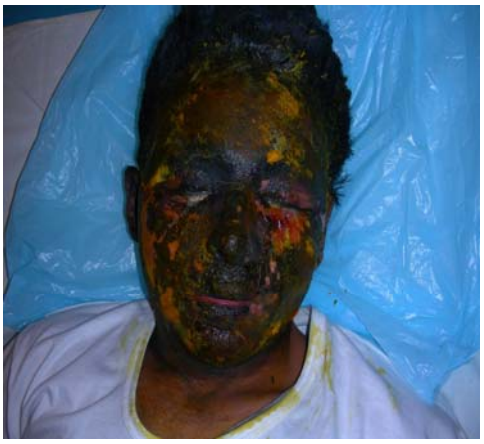
b

c

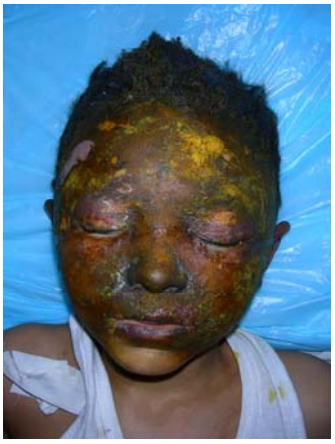


d

Deep partial-thickness burn: a, b, c, d-after complex treatment of "Camelyn M-3" "Camelyn M-2" and "Camelyn M-1"



a



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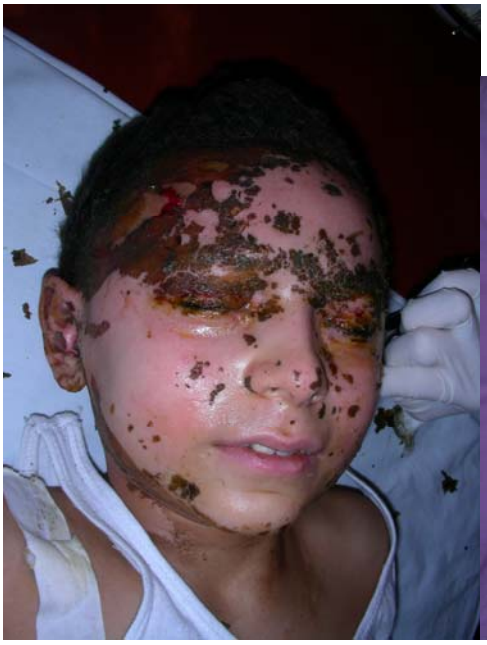

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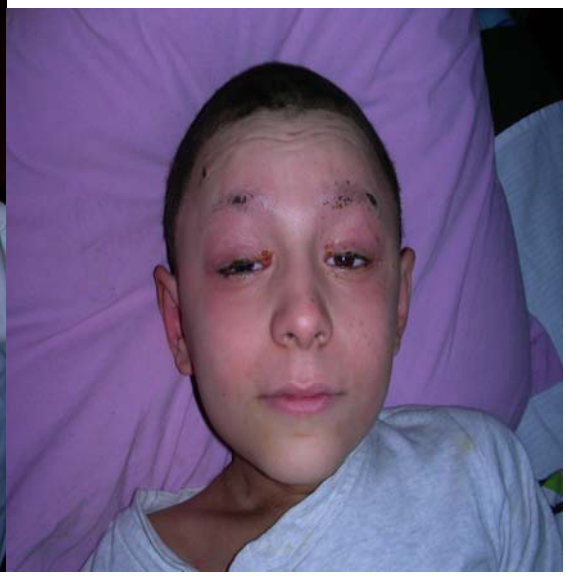


d

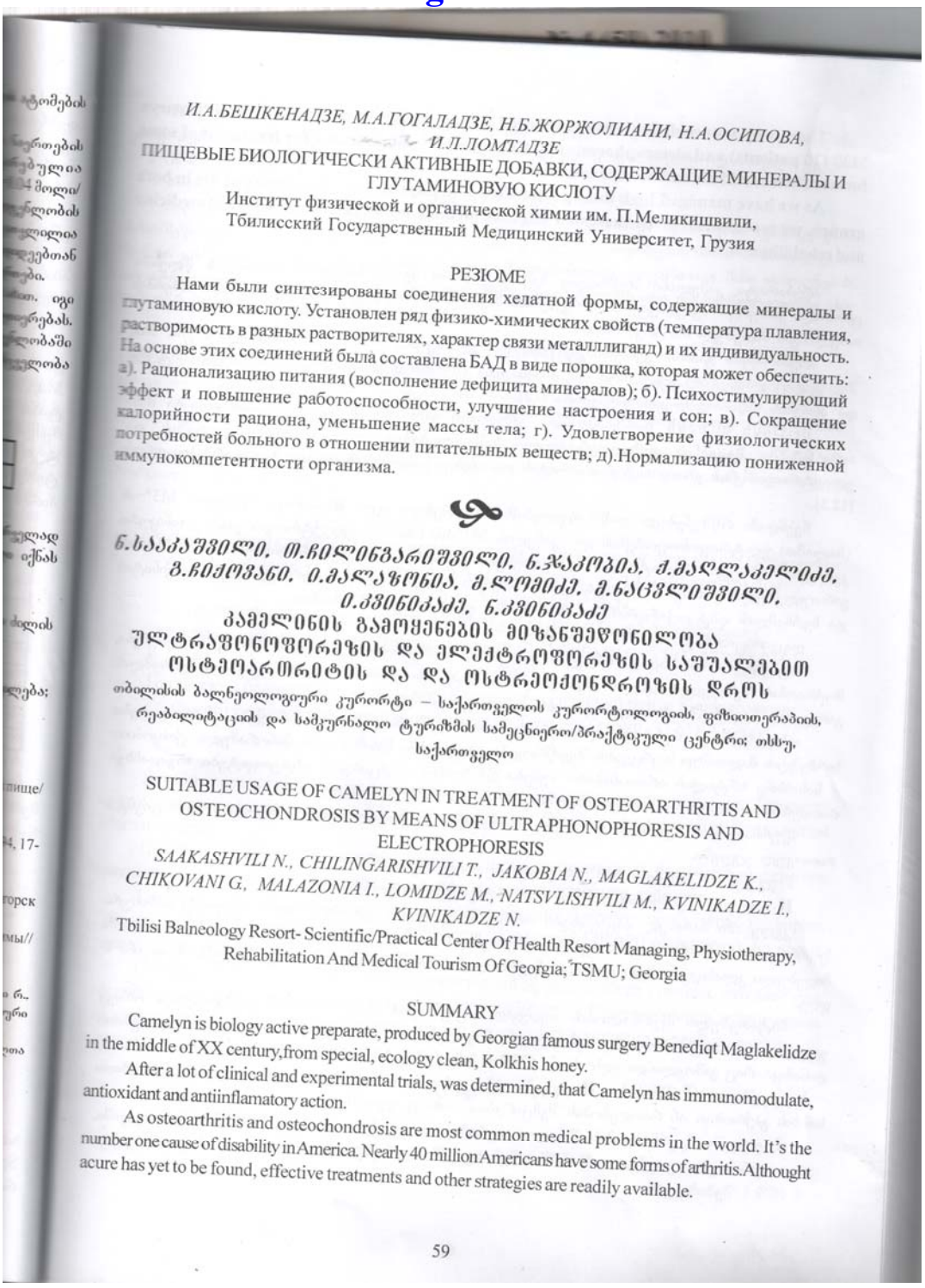
Full-thickness burns from structure fire: a-b-c-d after complex treatment of "Camelyn M-3", "Camelyn M-2" and "Camelyn M-1"

Clinical-laboratory and immunological studies allow us to safely recommend medicines of "Camelyn" for active use for rapidly treatment of thermal damage and pathogenetic treatment of patients with burn trauma.

Article Summary

"Suitable usage of preparations of Camelyn in treatment ofosteoartritis and osteochondrosis by means of ultraphonophoresis and electroforesis "- Experimental and Clinical Medicine, #4, (59), 2010, pp:59-62

Tbilisi Balneology Resort-Scientific/Practical Center of Health Resort Managing, Physiotherapy, Rehabilitation and Medical Tourism of Georgia.



И.А.БЕШКЕНАДЗЕ, М.А.ГОГАЛАДЗЕ, Н.Б.ЖОРЖОЛИАНИ, Н.А.ОСИПОВА, И.Л.ЛОМТАДЗЕ

ПИЩЕВЫЕ БИОЛОГИЧЕСКИ АКТИВНЫЕ ДОБАВКИ, СОДЕРЖАЩИЕ МИНЕРАЛЫ И ГЛУТАМИНОВУЮ КИСЛОТУ

Институт физической и органической химии им. П.Меликишвили, Тбилисский Государственный Медицинский Университет, Грузия

РЕЗЮМЕ

Нами были синтезированы соединения хелатной формы, содержащие минералы и глутаминовую кислоту. Установлен ряд физико-химических свойств (температура плавления, растворимость в разных растворителях, характер связи металлligанд) и их индивидуальность. На основе этих соединений была составлена БАД в виде порошка, которая может обеспечить: а). Рационализацию питания (восполнение дефицита минералов); б). Психостимулирующий эффект и повышение работоспособности, улучшение настроения и сон; в). Сокращение калорийности рациона, уменьшение массы тела; г). Удовлетворение физиологических потребностей больного в отношении питательных веществ; д).Нормализацию пониженной иммунокомпетентности организма.

ნ.საკაშვილი, თ.ჩილინგარიშვილი, ნ.ჯაკობია, ქ.მაღლაკელიძე, გ.ჩიკოვანი, ი.მალაზონია, მ.ლომიძე, მ.ნაცვლიშვილი, ი.კვინიკაძე, ნ.კვინიკაძე

კამელინის გამოყენების მიზანშეწონილობა შლტრაფონოფორეზის და ელექტროფორეზის საშუალებით ოსტეოართრიტის და და ოსტრეოქონდროზის დროს

თბილისის ბალნეოლოგიური კურორტი – საქართველოს კურორტოლოგიის, ფიზიოთერაპიის, რეაბილიტაციის და სამკურნალო ტურიზმის სამეცნიერო/პრაქტიკული ცენტრი; თსსუ, საქართველო

SUITABLE USAGE OF CAMELYN IN TREATMENT OF OSTEOARTHRITIS AND OSTEOCHONDROSIS BY MEANS OF ULTRAPHONOPHORESIS AND ELECTROPHORESIS

SAAKASHVILI N., CHILINGARISHVILI T., JAKOBIA N., MAGLAKELIDZE K., CHIKOVANI G., MALAZONIA I., LOMIDZE M., NATSVLISHVILI M., KVINIKADZE I., KVINIKADZE N.

Tbilisi Balneology Resort- Scientific/Practical Center Of Health Resort Managing, Physiotherapy, Rehabilitation And Medical Tourism Of Georgia; TSMU; Georgia

SUMMARY

Camelyn is biology active preparate, produced by Georgian famous surgery Benediqt Maglakelidze in the middle of XX century,from special, ecology clean, Kolkhis honey.

After a lot of clinical and experimental trials, was determined, that Camelyn has immunomodulate, antioxidant and antiinflamatory action.

As osteoarthritis and osteochondrosis are most common medical problems in the world. It's the number one cause of disability in America. Nearly 40 million Americans have some forms of arthritis.Althought acure has yet to be found, effective treatments and other strategies are readily available.

59

That's why, we decided to consider the need of usage of the ultraphonophoresis of "Cam M3" (20 patients) and electrophoresis of "Camelyn MI" (20 patients), for treatment of forms of osteoarthritis and osteochondrosis.

As we have managed high therapeutic effect and improving of the quality of life groups, we recommend to use these new effective methods widly in clinical, physical and rehabilitation.

პრეპარატი კამელინი შექმნილია საქართველოში მე-20 საუკუნის ორმოციან ცნობილი ქირურგის ბენედიქტე მაღლაკელიძის მიერ. ის წარმოადგენს განსაკუთრებული თაფლისგან მიღებულ ბიოპრეპარატს.

პრეპარატმა გაიარა ექსპერიმენტული და კლინიკური კვლევების ხანგრძლივი შედეგადაც დადგენილ იქნა, რომ მას ახასიათებს იმუნომოდულატორული, ანტიო და ანთების საწინააღმდეგო მოქმედება [1,2].

შრომის მიზანს წარმოადგენდა კამელინის გამოყენების სფეროს გაფართო ორგანიზმში შეყვანის ფიზიოთერაპიული საშუალებებით – ულტრაფონოფორე ელექტროფორეზის გზით, ოსტეოართრიტის და ოსტეოქონდროზის სხვადასხვა ფორმ [1,2,3].

ჩვენდამი რწმუნებულ დაწესებულებაში ჩატარებულ იქნა პრეპარატ "კამელინ (მალამო) ულტრაფონოფორეზის და "კამელინ M1"-ის (ხსნარი) ელექტროფორეზის კვლევა, შემდეგი რევმატიული დაავადებების დროს – ოსტეოართრიტები, მათ გართულებული ფორმებით (რეაქტიული სინოვიტი, ბეიკერის ცისტა, ბურსიტი, ტენდო და ხერხემლის ოსტეოქონდროზები, მათ შორის ფესვობრივი სინდრომით.

დაკვირვება ჩატარდა 40 პაციენტზე.

პაციენტებს ძირითადად შემდეგი ჩივილები აღენიშნებოდათ: ტკივილი სახსრებში, მოძრაობის შეზღუდვა, დილის შებოჭილობა. ოსტეოქონდროზის დროს ტკივილი გარკვეულ სეგმენტში, ზოგჯერ დადებითი ლასეგის სიმპტომი.

ობიექტურად: რენტგენოლოგიურად– სასახსრე ნაპრალების შევიწროვება, ოსტ ხერხემლის მალთაშუა სივრცეების შევიწროვება, კაუჭისებრი წანაზარდები. პანორამული – სასახსრე ხრტილის არათანაბარი შენება და ზომაში შემცირება, ოსტეოფიტები, რაოდენობით ანთებითი სითხე. სისხლში ედს-ის და CRP-ს მომატება.

მკურნალობის მეთოდის მიხედვით პაციენტები დაყოფილ იქნენ 2 ჯგუფად თითოეულ ჯგუფში.

I ჯგუფი – "კამელინ M3"-ის ულტრაფონოფორეზი.

II ჯგუფი – "კამელინ M1"-ის (ხსნარი) ელექტროფორეზი.

კვლევაში ჩართულ პაციენტებს ორივე პროცედურა უტარდებოდათ როგორც მკურნალობა, დაზიანებულ სახსრებზე და ხერხემლის დაინტერესებულ სეგმენტზე, ფიზი მიღებული კლასიკური მეთოდიკებით. მკურნალობის ხანგრძლივობამ ორივე ჯგუფში დღე.

ჩატარებული მკურნალობის შედეგად პაციენტების მდგომარეობა გაუმჯობ ჯგუფში, გამოიხატა როგორც სუბიექტური ჩივილების, ისე ობიექტური მონაცემების დინამიკა, რაც გამოვლინდა ედს-ის და CRP-ს მაჩვენებლების სარწმუნო დაქვეითებით – **I** ჯგუფში და ($P<0,001$) – **II** ჯგუფში, ექოსკოპიური მონაცემების გაუმჯობესებით სითხის გაქრობით ან რაოდენობის შემცირებით) ორივე ჯგუფში.

I ჯგუფში მკურნალობის მაღალი თერაპიული ეფექტი მივიღეთ 7 (35%) კარგი თერაპიული ეფექტი – 10 (50%) შემთხვევაში, დამაკმაყოფილებელი თერაპ – 3 (15%) შემთხვევაში.

Food Preservation

Scientific-Pharmaceutical Company “Camelyn”

Food preservation with “Camelyn M”is the process of treating and handling food in such a way as to stop or greatly slow down spoilage to prevent foodborne illness while maintaining nutritional value, density, texture and flavor.

Preservation wit “Camelyn M” involves preventing the growth of bacteria, fungi and other micro-organisms, as well as retarding the oxidation of fats which cause rancidity. It also includes processes to inhibit natural aging and discolouration that can occur during food preparation such as the enzymatic browning reaction for egzample in eggplants and apples which causes browning when they are cut.

1.

2.

Fig1. Package eggplants in presence with 0,01ml solution of Camelyn and Fig2. Control after 30 day of incubation on the room temperature (28-31 ^{0} C)

The same case after opening a package with eggplants

SUMMARY

The preparation was tested against various form of malignant tumor and Infection deceases in several leading clinics of Moskow, Leningrad, Tbilisi, Batumi, Quebec, Montreal, Chicago, Bern, Rio de Janeiro, with very promising results. In the beginning of 2006, the Drugs Department of the Ministry of Labor, Health and Social Security registered the preparation. Registration certificatesm, numbers: DR No h-001045, DR No h-001046, DR No h-001047, DA #R-003862.

Pharmacological forms

- 35% solution of "Camelyn M1" for injections in 2 ml ampoules, 10 ampoules in each box.

- Capsules of "Camelyn M2" containing 0.5 g of dry substance, 30 capsules in each package, 10 capsules in each blister pack.

- "Camelyn M3" ointment in 25 g tubes.

-"Camelyn M4" rectal vaginal suppositories 2.0g #10

Solution, powder, ointment and suppositories of "Camelyn M" is used for both children and adults, as a component of complex therapy, and independently in cases where stimulation of immunity and modification of immune response is necessary also:

· Bacterial infections
· Virus infections
· Fungous infections
· Polyneiropathy
· Degeneration-dystrophic diseases of anchylosis
· Tumor diseases
· Post operation period
· Traumas

Urologic Desease(chronic bactericidal prostatitis, chronic urethritis)

Osteoarthritis, Spinal osteochondrosis

Has an anti-inflammatory, regenerative, analgesic effect. Purulent, prolonged unhealed wounds of soft tissues and skin, trophic ulcers, panarycias, burns, bedsores.

Rectal preparation of "Camelyn M-4" are ideal for young infants, debilitated patients, or those who can not tolerate oral intake. This preparations can be used for local administration for treatment of hemorrhoids, itching, inflammation, and many rectal and vaginal infections and diseases.

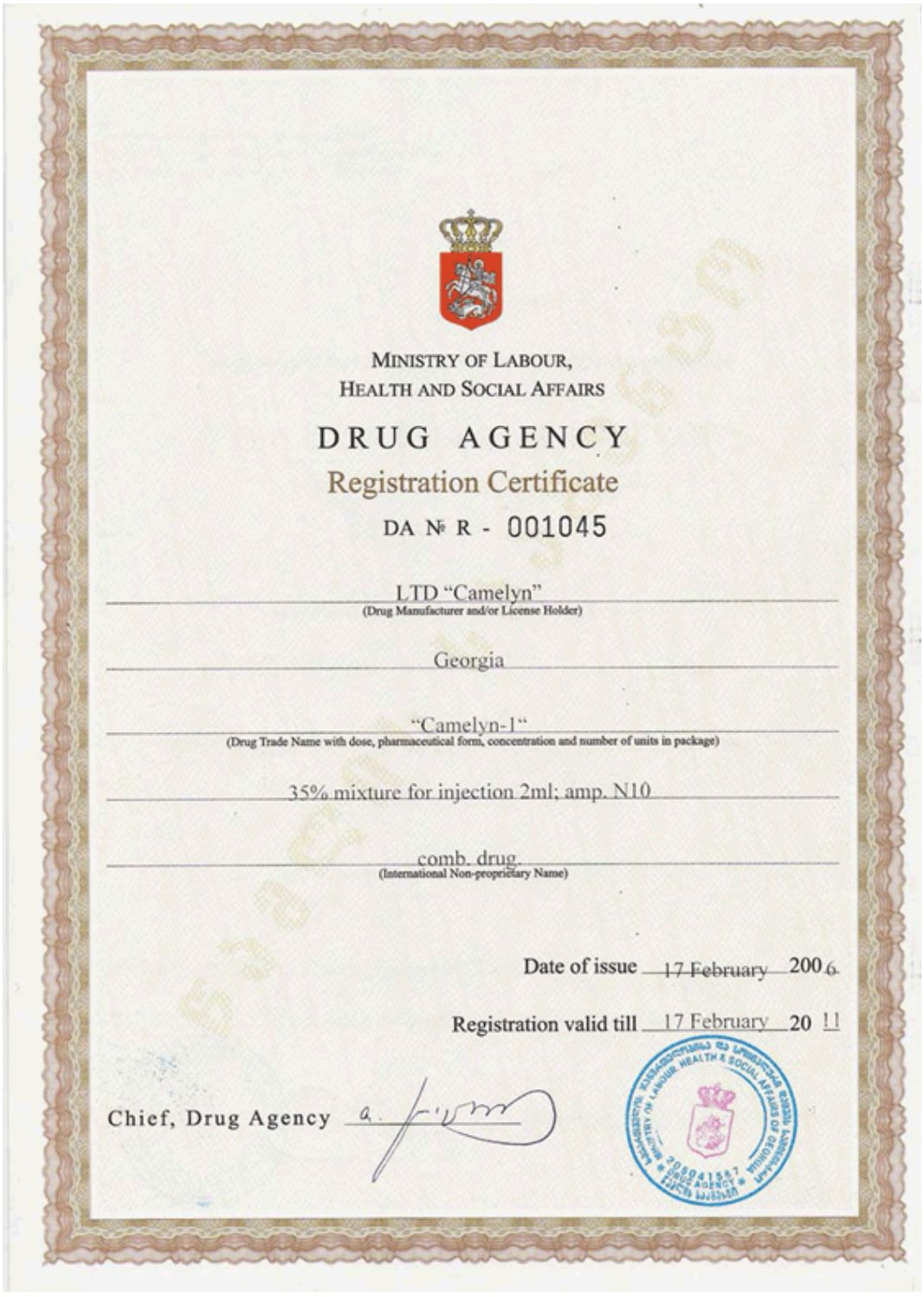

CAMELYN - M1
2ml


CAMELYN - M2


7, KINDZMARAULI STR,
TBILISI, GEORGIA. CAMELYN LTD
CAMELYN - M4
[CAMELINUM]
10 SUPPOSITORIES
2.0g
CAMELYN
MADE IN GEORGIA
КАМЕЛИН М4
1 УПАКОВКА -10 СВЕЧЕЙ
ООО "КАМЕЛИН"


CAMELYN M-3

CERTIFICATES



MINISTRY OF LABOUR,
HEALTH AND SOCIAL AFFAIRS

DRUG AGENCY

Registration Certificate

DA № R - 001045

LTD "Camelyn"
(Drug Manufacturer and/or License Holder)

Georgia

"Camelyn-1"
(Drug Trade Name with dose, pharmaceutical form, concentration and number of units in package)

35% mixture for injection 2ml; amp. N10

comb. drug.
(International Non-proprietary Name)

Date of issue 17 February 2006

Registration valid till 17 February 20 11

Chief, Drug Agency

MINISTRY OF LABOUR,
HEALTH AND SOCIAL AFFAIRS

DRUG AGENCY

Registration Certificate

DA № R - 001046

LTD "Camelyn"
(Drug Manufacturer and/or License Holder)

Georgia

"Camelyn-2"
(Drug Trade Name with dose, pharmaceutical form, concentration and number of units in package)

0.5g capsule N30

comb. drug.
(International Non-proprietary Name)

Date of issue 17 February 2006

Registration valid till 17 February 2011

Chief, Drug Agency

MINISTRY OF LABOUR,
HEALTH AND SOCIAL AFFAIRS

DRUG AGENCY

Registration Certificate

DA № R - 001047

LTD "Camelyn"
(Drug Manufacturer and/or License Holder)

Georgia

"Camelyn-3"
(Drug Trade Name with dose, pharmaceutical form, concentration and number of units in package)

5% ointment 25g in tube

comb. drug.
(International Non-proprietary Name)

Date of issue 17 February 2006

Registration valid till 17 February 2011

Chief, Drug Agency

Approved by Minister of Labour, Health and Social Affairs of Georgia
16.10 2006 Order 269/6

MINISTRY OF LABOUR,
HEALTH AND SOCIAL AFFAIRS OF GEORGIA

DRUG AGENCY

Registration Certificate

DA № R - 003862

"Camelyn" Ltd
(Pharmaceutical Product Manufacturer or License Holder, producing country)

Georgia

"Camelyn M4"
(Product Trade Name, dosage and pharmaceutical form, concentration and number of units in package)

Rectal vaginal suppositories 2.0g N10

(International Non-proprietary Name (INN)

Date of Registration: 29 January 2008

Registration valid till: 17 February 2011

Head of Drug Agency _____ Seal

References:

ANTICANCER EFFECTS OF PREPARATE "CAMELYN" OBTAINED FROM HONEY (in press 2008)

B.Maglakelidze, K.Gurielidze, Vakhtang Mshvildadze* and Vinsent Perreten*
Scientific-Industrial Company "Camelyn M"; 7 Kindzmarauli str, Tbilisi, Georgia
*Laboratoire LASEVE, Universiter du Querbec a` Chicoutimi 555, Boulevard de l'Universiter, Chicoutimi, Querbec, Canada G7H2B1
Address for correspondence: Scientific-Industrial Company "Camelyn M"; 7 Kindzmarauli str, Tbilisi, Georgia; e-mail: kgurielidze@camelyn.com

Summary: The effects of therapy with "Camelyn", active compound obtained from special sort of honey in one of region of Georgia, were examined in 15500 cancer patients, between 1965 and 2003, on the III and IV stage of disease, mainly on the terminal stage of deeply lying tumors of internal organs. "Camelyn" is a mixture of different biologically active compounds: albumins, peptides, amino acids, aldehydes, furfural, formic acid, microelements. Significant anticancer activity by "Camelyn" was observed in patients given intramuscularly 5 g of "Camelyn" 3 times per day with Novocain. Number of patients with wearies cancer decease were as follows: 1.Carcinoma of prostate gland-2000; 2.Carcinoma of uterine cervix-1200; 3.Ovarian carcinoma-1800; 4.Carcinoma of urinary bladder-2000; 5.Renal tumor-1000; 6.Colon cancer-3000*;* 7. Fibrosarcoma-500; 8.Carcinoma of stomach-1500*,* 9. Lung cancer-500; 10.Oesophagus cancer-1000; : 11.Larynx tumor-500; 12. Endothelioma-500. "Camelyn" displays a strong inhibiting action on the growth of certain forms of tumors in experiment, as well as in clinic, to the point of their full regression.

Two mechanisms by which "Camelyn" exerts its effect were investigated. The first was immunomodulation. The mechanism of action involves the stimulation of three important cytokines: IL-2, IFN-γ, and TNF-α. The second was direct anticancer properties. *In vitro* experiments, "Camelin" was found to be active against DLD-1 colon carcinoma and A-549 lung carcinoma cells, with IC50 values of 0.063 ± 0.006 μg/mL and 0.078 ± 0.005 μg/mL , respectively.I*n vivo* studies in mice showed that "Camelyn" possesses suppressive effects on tumour cell growth, 0.1 ml 35% solution of "Camelyn caused full regression of tumor in maic. It is concluded that the high anticancer effect of "Camelyn" and the absence of notable side-effects make "Camelyn" a promising therapeutic agent for the treatment of cancer patients. At the same time, the preparation stimulates growth and reproduction of young connective tissues.

Introduction

Honey's natural components role as a biologically active compound has been well documented for over 40 years. Numerous studies have subsequently shown that hone exhibit immunostimulating properties, including antibacterial and anti-tumour activities (1, 2 3,4). The therapeutic properties of "Camelyn" have been studied by us from 1946. "Camelin" can be isolated from special species of honey from one of region of Georgia (6). "Camelyn" is a mixture of different biologically active compounds: albumins, peptides, amino acids, aldehydes, furfural, formic acid, microelements(6). "Camelyn" derived from honey has been the most extensively studied in Georgia and Russia. (6) Different physicochemical parameters of Camelin's compounds, such as solubility, primary structure, molecular weight, and other properties play a significant role in the biological activities of "Camelin" (6). Subsequent studies demonstrated that "Camelin" has strong antibacterial activity in a wide variety of bacterial species, including staphylococcus, colon bacillus, blue pus bacillus, typhus-paratyphoid group microbes, dysentery bacillus, *Mycobacterium tuberculosis* and *B.antracis* (under investigation). More than 15 publications have reported that "Camelin" is non-toxic preparation, in addition, numerous of concentrations and routes of administration have been tested in mice, guinea-pigs, dogs and humans during more than 60 years of investigation, including oral, intraperitoneal, subcutaneous, and intravenous applications (6). Based on these results it has been concluded that in "Camelin" (mixture of natural compounds of honey), represents a type of biologically active compounds, likely representing an evolutionarily-conserved innate defence response directed against bacterial, viral and fungal pathogens (9).

The theory of immune surveillance postulates that immune effectors can recognize and destroy spontaneously arising malignant tumour cells. Tumours may develop when transformed cells escape immunological host defence mechanisms. The increased incidence of spontaneous tumours in immunosuppressed individuals, as well as those with congenital or acquired immunodeficiencies, indicates that the immune system can provide a major mechanism for host resistance against cancer and

infectious diseases (10-GHONEUM M. et all). GHONEUM M. et all demonstrated that active hemicellulose compound (AHCC) is a effective anticancer agent that does not have any known side-effects. The mechanism(s) by which AHCC exerts its anticancer activity may be through: (i) NK cell immunomodulation; (ii) direct anticancer activity.

In this paper we report on the action and mechanism *(in vitro* and *in vivo* in mice cancer model) of anticancer activity of "Camelin" in 1400 cancer patients on the III and IV stage of disease, mainly on the terminal stage of deeply lying tumors of internal organs. In this study we tested the effects of subcutaneous administration of pharmaceutical form of "Camelin M1" on tumor growth in cancer patients.

Animals, Patients, Materials and Methods:

Mice

Female, 6-wk-old BALB/c mice were purchased from Institute of Experimental and Clinical Surgery, Tbilisi, Georgia. Care and handling of mice followed guidelines set out by the Georgian Council on Animal Care.

***In vitro* assay:**

Cell culture

The human lung carcinoma A-549 (ATCC #CCL-185), colon adenocarcinoma DLD-1 (ATCC #CCL-221), and murine macrophage RAW 264.7 (ATCC #TIB-71) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, USA). The A-549, DLD-1 cell lines were grown in Minimum Essential Medium with Earle's salts, while the RAW 264.7 cell line was grown in Dulbecco's modified Eagle's medium (Mediatech Cellgro®, Herndon, USA). Both media were supplemented with 10% fetal calf serum (Hyclone, Logan, USA), solution of vitamins (1X), sodium pyruvate (1X), non-essential amino acids (1X), penicillin (100 IU) and streptomycin (100 □g/ml) (Mediatech Cellgro®). Cells were cultured in a humidified atmosphere at 37 ºC in 5% CO_2.

Cytotoxicity assay

Exponentially growing cells were plated at a density of 5 x 10^3 cells per well in 96-well microplates (Costar, Corning inc.) in 100 µl of culture medium and were allowed to adhere for 16 hours before treatment. Then, 100 µl of increasing concentrations of extract or pure compounds dissolved in the appropriate solvent (Sigma-Aldrich) were added. The final concentration of solvent in the culture medium was maintained at 0.5% (volume/volume) to avoid solvent toxicity. The cells were incubated for 48 h in the absence or in the presence of extract. Cytotoxicity was assessed using the resazurin reduction test as described by O'Brien (O'Brien et al., 2000). Fluorescence was measured on an automated 96-well Fluoroskan Ascent Fl™ plate reader (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxicity was expressed as the concentration of extract or compound inhibiting cell growth by 50% (IC_{50}).

***In vivo* assay**

Tumour cell-lines

Mouse M-1 sarcoma line cells was passaged *in vivo* in BALB/c mice. Twenty-one days after inoculation, the tumors were excised, gently teased over stainless steel screens, washed in RPMI 1640 medium and resuspended in PBS (1x106 viable cells/ml).

Patients

With the purpose of studying the effect of Camelyn during treatment of the patients suffering from malignant tumors, we have selected 1400 patients on the III and IV stage of disease, mainly on the terminal stage of deeply lying tumors of internal organs (incurable cases). Experiments we are carried out, at the Tbilisi State Medical Institute, Leningrad Institute of Oncology of the Academy of Sciences of the USSR and at the Moscow Institute of the Experimental Pathology and Therapy of Tumor, between 1965 and 2003. Patients were treated with "Camelin" after conventional therapies such as surgery, chemotherapy and radiation. All patients had a pathological report confirming a diagnosis indicating the type and stage of cancer. The type of chemotherapy was obtained from medical records. Number of patients with wearies cancer decease in the study were as follows: 1.Carcinoma of prostate gland-2000; 2.Carcinoma of uterine cervix-1200; 3.Ovarian carcinoma-1800; 4.Carcinoma of urinary bladder-2000;

5.Renal tumor-1000; 6.Colon cancer-3000; 7. Fibrosarcoma-500; 8.Carcinoma of stomach-1500, 9. Lung cancer-500; 10.Oesophagus cancer-1000; : 11.Larynx tumor-500; 12. Endothelioma. There were one case history in of each types of cancers patients (see table 1.).
This study was approved by the Tbilisi State Medical Institute, Leningrad Institute of Oncology of the Academy of Sciences of the USSR and at the Moscow Institute of the Experimental Pathology and Therapy of Tumor Institutional Review Board (Medicine), and all of the patients signed an approved informed consent.

Material

The solvents were purchased from EMD (Canada).
Fetal calf serum and RPMI 1640 medium were purchased from Gibco BRL (Rockville, MD), HEPES, Novocaine, Concanavalin A, All other reagents were purchased from Sigma–Aldrich.

Cytokines

For evaluation of cytokine production, we incubated purified spleen cells from each animal (2x106 cells/ml in RPMI 1640 medium with 5% FCS) in wells of a 24-well tissue culture plate. After addition of 1 μg of Concanavalin A or 10 μg of LPS per well, cells were incubated for 72 hr in a humidified incubator (37°C, 5% CO2). At the endpoint of incubation, supernatants were collected, filtered through 0.45 μm filter and tested for the presence of IL-2, IFN-γ and TNF-α. Levels of cytokines were measured by commercial ELISA in accordance with the protocol for Cytoscreen™ of BioSource International, Inc. (Camarillo, CA) .

The preparation Camelyn was obtained in the liquid form from the special sort of honey (5).
PH=3-4 dilution 1:4, 1:8 does not change pH.

Table 1. Histological diagnosis of 14 cancer patients and type of conventional therapy.

Case istory #	Patient	Age years	Sex	Type of cancer	Treatment	Condition of patients	
						After treatment of Camelin	Before treatment of Camelin
5805	A.	13	M	Endothelioma in cervical area	Surgery* Radiation* Camelin**	Very heavy	In good health
222	D.	41	M	Oesophagus cancer with metastasis in ileocecal area	Surgery* Chemo* Camelin**	Very heavy	In good health
7709	K.	43	F	Larynx tumor, squamous cell epithelial cancer with keratinization	Surgery* Chemo* Camelin**	Very heavy	In good health
263	G.	65	M	Lung cancer	Surgery* Chemo* Camelin**	Very heavy	In good health

12851	V.	4	M	Sarcoma on the parietal lobe, fusocellular sarcoma see fige 2	Surgery* Chemo* Camelin**	Very heavy	In good health
346	K.	42	F	Sarcoma of sacral part See fige 3	Surgery* Chemo* Camelin**	Very heavy	In good health
3478	G.	12	M	Sarcoma of the left femur, fibrosarcoma	Surgery* Chemo* Camelin**	Very heavy	In good health
895	M.	51	M	Sarcoma of stomach, Reticuloblastoma	Surgery* Chemo* Camelin**	Very heavy	In good health
644	R.	25	M	Colon cancer, adenocarcinoma	Surgery* Chemo* Camelin**	Very heavy	In good health
7239	M.	48	F	Renal tumors, malignant hypernephroma	Surgery* Chemo* Camelin**	Very heavy	In good health
868	B.	65	M	Carcinoma of the urinary bladder	Surgery* Chemo* Camelin**	Very heavy	In good health
6701	D.	60	M	Carcinoma of prostate gland, metastasis on the right ischial bone	Surgery* Chemo* Camelin**	Very heavy	In good health
5421	A.	48	M	Carcinoma of uterine cervix, adenocarcinoma	Surgery* Chemo* Camelin**	Very heavy	In good health
4359/261	M.	29	F	Ovarian carcinoma	Surgery* Chemo* Camelin**	Very heavy	In good health

*-Means treatment was before Camelyn therapy; **- Means treatment is continued

Method

Tests in mice were carried out using of 0,1 ml dose of 35% solution of the preparation Camelyn on 100,0 g weight of the animal ( ). The preparation was introduced intraperitoneally every day during two weeks. Tumours were measured every 4th day, calculation of average diameter of tumours was performed by the following formula:

$$D = \frac{a+b+c}{3}$$

Where:
D – average diameter of the tumour,
a - length of the tumour,
b – width of the tumour,
c - height of the tumour.
Growth inhibition of the tumour was calculated by the following formula:

$$t = \frac{MB - MO}{MB} \times 100$$

Where:
MB - is an average weight of the tumour in control,
MO – is an average weight of the tumour during tests.

Treatment protocol- In patients, the preparation Camelyn was injected intramuscularly in rising doses from 1 to 5 g 3 times per day with Novocaine. At first 2-3 g 0,5-1% solution of Novocain was administered, accuse is left during 2-3 minutes, after the preparation was added reaching 5 g, duration of treatment 5-6 days, further gradually is administered 2-3 g.

3. Results and Discussion

3.1. Evaluation of cytotoxicity against tumor cell lines

The anticancer activity of both 35%; 100% solution and powder (dried) form of preparation of "Camelin" was evaluated against colon carcinoma cell line DLD-1 and lung carcinoma cell line A-549. As shown in Table 1. the powder form of "Camelin" was inactive against DLD-1 and A-549 with an IC50 value higher than 200 µg/ml. In contrast, the results presented in table 1. show that the 35% and 100% solution of "Camelin" was active against A-549 and DLD-1, with IC50 value of 0.063 ± 0.006 µg/ml; 0.078 ± 0.005 µg/ml and 0.070 ± 0.004 µg/ml; 0.104 ± 0.002 µg/ml, respectively.

Table 1. Cytotoxic effect of 35% ;100% solution and powder (dried) form of preparation of "Camelin" against carcinoma colon cell lines DLD-1 and lung adenocarcinoma cell lines (A-549).

Cancer cells	A-549	DLD-1
Camelin-Powder	>200 µg/mL	>200 µg/mL
Camelin -M1 35%	0.063 ± 0.006 µg/mL	0.078 ± 0.005 µg/mL
Camelin-SBS-100%	0.070 ± 0.004 µg/mL	0.104 ± 0.002 µg/mL

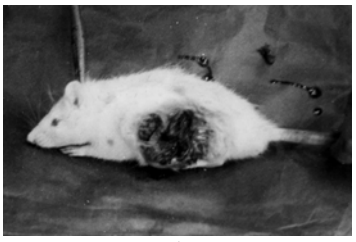
As it follows from the Table 2. establishment of tumors after their inoculation in mice in all cases accounts for about 80-90%.

Table 2.
Peculiarities of the Sarcoma M-1 growth Transplanted After inoculation of tumor and treatment with Camelin in mice

Group	Inoculation of tumor on the 10th day				Treatment with Camelyn					
					After 20 days	After 1,5 month				
	Number of animals	Was inoculated	Wasn't inoculated	%	Size of tumor (mm)	Disappeared	Remained	%	Size of tumor (mm)	Died due to tumor
I	10	8	2	80	5,7	7	1	87.5	0,2	
II	10	9	1	90	3,4	9	0	100	0	
III	10	8	2	80	7	8	0	100	0	
IV	9	5	4	55,5	6,6	4	0	100	0,4	
V	10	8	2	80	6,4	8	0	100	0	
Control	25	20	5	80	17,8	-	-	-	-	20 rats

As it follows from the Table 2, inoculation of tumors (Fig: 1-a,b and c) in the control, as well as in the test groups, is about the same, though in this case the size of tumors after 20 days of treatmen twith Camelin in test groups is significantly less, than in the control (Table 2.). After 1,5 months considerable part of tumors in testanimals resorpted (Table 2; Figure 1-d and e) , when in the control group the tumors continued to grow and all the animals died.


a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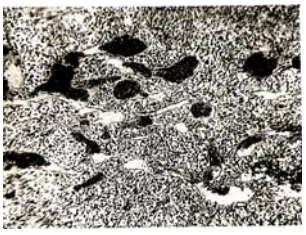

c


d

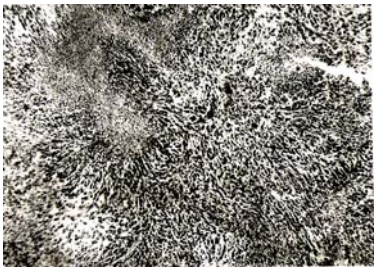

e

Fig 1. Rat sarcoma before and after treatment with preparation of Camelin
a,b-infected mice from control group; c- histomorphological picture of tumor in control mice.
d-mice from experimental group after1,5 months (tumor resorpted); e- histomorphological picture of tumor in experimental mice (tumor resorpted).

Investigation of the effect of oral introduction of the preparation on the tumor growth was interesting. For clarification of the issue we performed the following tests: 30 rats were tested: 10 of which constituted the 1st group, 10-II and 10 – the control. To the rats of the first group during 10 days one time a day was given 0,5 ml of the 10% solution of Camelyn, after which sarcoma M-1 was inoculated to them. (Table 3)

To the rats of group II in the same way during 10 days was given the preparation in the same doze, following to which sarcoma M-1 was inoculated to them. After inoculation of the tumor the animals continued to receive Camelyn in the same doze one time a day during 40 days (Table 3).
Consequently, in the organism preliminarily prepared with Camelyn, the tumor cells loose considerably their malignant properties and the tumors resorpt. This is clearly seen, if introduction of the preparation is continued during a long time after inoculation.

These observations brought us to the following the working assumption of the mechanism of Camelyn action. Under the effect of Camelyn malignancy of the tumor cells is reduced, at that we could think that this effect depends on some changes in the antigen composition of tumors leading to the changes of immunobiological reactivity of the organism. Consequently, the tumors processed with Camelyn regress, that, in its part, causes the immunobiological shifts in the organism, creating the condition of resistance to the given tumor strain.

Table 3.

Peculiarities of the Sarcoma M-1 growth during oral introduction of Camelin in mice

Group	Duration of Introduction of the Preparation, days	Inoculation of tumor on the 10th day					Resorption after 1,5 month				
		Number of animals	Was inoculated	Wasn't inoculated	%	Size of tumor, mm	Disappeared	Remained	%	Size of tumor, mm	Died due to tumor
I	10	10	5 3 8	5 7 2	50 30 80	6 3 17,	3 2 -	2 1 8	60 66,6 -	3,6 0,6 28,3	
II	40	10				3					
Control	-	10									8 rats

Evidence of orally-administered Camelin immunomodulatory activity was also demonstrated through effects on the production of three different cytokines, IL-2, IFN-□, and TNF-□ (Table 4). The production of these three cytokines was measured after a 72 hr *in vitro* incubation of spleen cells isolated from control and Camelin administered animals. For all three tested cytokines, oral administration of Camelin resulted in significantly-increased cytokine levels IL-2 (3.1-fold), IFN-γ (4.4-fold), and TNF-α (8.7-fold), $P < 0.05$) over control animals.

Table 4. *in vivo* Effects of oral administration of Camelin on cytokines

Cytokine	Control	Camelin
IL-2	14.5+/- 0.7 pg/ml	45.6 +/- 4.1 pg/ml*
IFN-γ	163.4 +/-12. 4 pg/ml	721.3 +/- 64.4 pg/ml*
TNF-(α)	362.2 +/- 43.2 pg/ml	3156.2 +/- 129.9 pg/ml*

*P values were determined using a student's T-test ($P<0.05$ for IL-2, IFN-γ, and TNF-α).

Many of cases were demonstrated on the united session of surgical, oncologic and urological societies of Georgia on February 7, 1968; March 16, 1975; April 1998 and Julay 3, 2003.
During analysis of the carried out observations first of all becomes clear that at present we have all the reasons for optimistic evaluation of antitumor properties of the preparation. Camelyn displays a strong

inhibiting action on the growth of certain forms of tumors in experiment, as well as in clinic, to the point of their full regression. As to the mechanism of the Camelyn action, this issue, apparently, needs further studies. But the given data enable to assume that Camelyn causes certain shifts in antigenic composition of tumors leading to the change of immunobiological reactivity of the organism and conditioning the state of resistance of the organism against the tumor growth.

It should be noted, that all the patients, with the exception of some – with larynx tumor, had far gone forms of disease, whereas some were operated many times in the past, one of them – 6 times.

In spite of the grave condition of patients, terminal stage of the disease, treatment with Camelyn appeared to be efficient. The patients were observed during the period beginning from a year to 10 years after treatment.

Among 1400 patients under observation relapse was marked among 40. For example: at carcinoma of the urinary bladder (case histories N 7743,) in 4 years after treatment the patient was hospitalized with a relapse, after which he died. The second (case histories N 7743,) – in 7 years after treatment went to the IInd urological department of the Central Republican Hospital in October – after the operation was discharged in good condition. The third (case histories N 7743,), suffering from Carcinoma of stomach , in 2 years after treatment had a relapse. At present he is being treated with Camelyn. The forth (case histories N 7743,) patient suffering from carcinoma of the prostate gland with metastasis of ischial bone and urine retention, following to the 5 year of treatment went to the 1st urological department of the Central Republican Hospital. The operation cystostomia was carried out, at opening of the urinary bladder a large size stone was found. The patient was discharged from the clinic in good condition.

Sometimes large tumors are resolved fully that, of course, improves general condition of the patient. In separate cases, carcinoma resolve only partially, in others – fully, even in the existence of larger tumors. Such cases are not many, but they are still marked. At Ovarian carcinoma, for example (case history #4359/261; see table 1), when the patient was operated 6 times, after each operation the tumor was relapsed in one and a half, two months. After the 6th operation the patient began administering of the preparation Camelyn. During the first course of treatment the tumor was resolved. Four years passed, the patient is healthy. There are no signs of relapse. The other patient (case history N 1285) suffering from sarcoma of parietal lobe with the defect of skull bone (fig 2.), was operated four times. After each operation the tumor grew again. During the first course of treatment with preparatin of Camelin, defect of the skull bone was restored. At present the patient is healthy; after the treatment 36 years have passed.

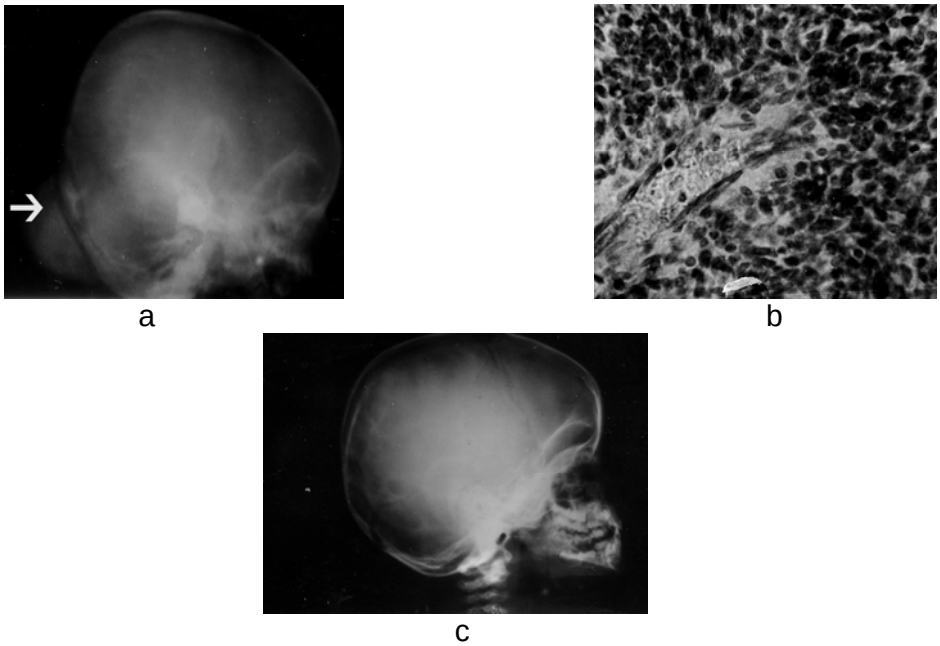
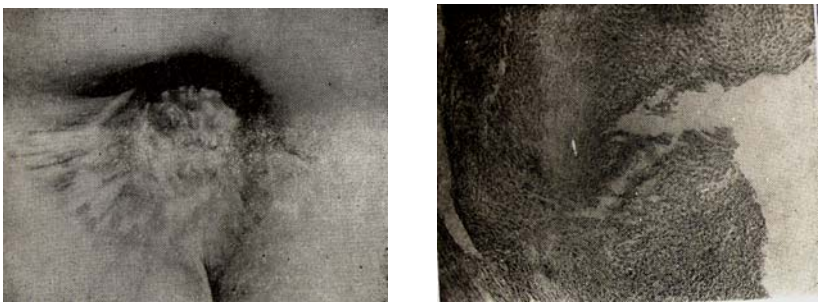
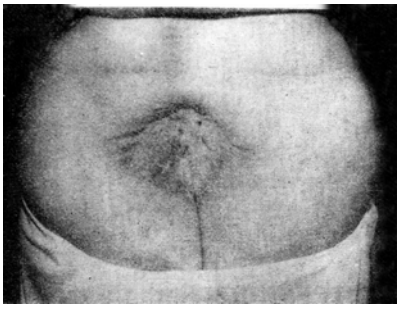


Fig.2. Defect of the skull before and after treatment of Cameline (5).



a b



c

Fig. 3. Sarcoma of sacral part before and after treatment of Cameline (5).
a- before treatment of Camelin; b- histomorphological analysis of the tumor
c- The same case after the third course of treatment of Camelin.

Of course, the above described cases of recovery constitute only a small part of patients with far gone forms of malignant growths treated with our preparation. In most cases, success was not achieved. But it should be noted, that almost in 100% of cases was obtained an effect in the form of improvement of general condition, temperature reduction, disappearance of pains, nausea, improvement of blood picture, reduction of tumor sizes, in separate cases resorption of metastasis, prolongation of life.
The obtained data speak for the necessity of the further experimental studies in this direction Especially in earlier stage of disease and raise a number of issues, solution of which requires a special laboratory environment and complex work of the different specialists.

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In Vitro Antifungal Activities of “Camelyn M” (in press 2008)

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Abstract

Candida species are an important cause of opportunistic infection in the oral cavity of immunocompromised patients, especially HIV infected patients. The activities of "Camelyn M", a mixture of natural compounds isolated from special sort of honey, distributed in one of region of Georgia, were evaluated *in vitro*. Camelyn M exhibited potent in vitro activities against fluconazole-resistant strains of *Candida albicans* , *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*, with MICs at which 90% of isolates were inhibited of 0.012 μg/ml, respectively.

Introduction

The risk of opportunistic fungal infections is greatly increasing in patients who are immunocompromised due to cancer chemotherapy, organ or bone marrow transplantation, or human immunodeficiency virus infection (1). *Candida albicans* is the organism most often associated with both mucosal and hematogenously disseminated infections (2, 3,4). Recently, azole-resistant *C. albicans* has become a clinical problem in AIDS patients with oropharyngeal candidiasis (OPC) and esophageal candidiasis (5, 6, 7, 8,9); and other *Candida* spp., such as *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei*, have emerged as clinically important pathogens (10, 11, 12). The oral azole antifungals clotrimazole, etoconazole, fluconazole and itraconazole are frequently used in patients who are HIV-positive as initial or suppressive therapy for oropharyngeal and esophageal candidiasis. Unfortunately, the incidence of fluconazole-refractory candidiasis is becoming increasingly more common and frequently may emerge during therapy in advanced HIVpositive patients [3,4]. Many of these patients they may suffer from frequent clinical relapses despite high doses of fluconazole and require parenteral amphotericin B. These overwhelming infections frequently impair the quality of life and may result in a reduction of fluid or food intake. Therefore, there is a need to search for novel antifungal agents that have novel modes of action and fewer side effects and that can be administered both orally and parenterally.
The therapeutic properties of "CamelinM" have been studied by us from 1976. Preclinical, clinical, double blind research and toxicity studies with "Camelin M" was performed in several clinical center of Moskow, Leningrad (Sankt Peterburg) and Tbilisi with very promising results. From 2004 "Camelyn M" in Georgia is an officially registered therapeutic, injection preparation, which is successfully used for treatment of malignant tumors (13).

"Camelyn M" acts by as detergent on the bacterial cell membranes and selectively inhibiting wearies of living mechanism of bacterial cells (13) . Apparently, these compounds are responsible for most of the antifungal and antimicrobial activity of honey.
In the present study, we investigated the in vitro antifungal activities of Camelyn M1 against pathogenic fungi.

Materials and methods

Antifungal agents. "Camelyn M" was isolated from honey as described previously (13) for the in vitro studies. For the in vitro study, fluconazole (FLC) and itraconazole (ITC) were extracted from commercial preparations purchased from PSP Pharmaceuticals, Inc. (Tbilisi, Georgia), and Aversi Pharma, Ltd. (Tbilisi, Georgia), respectively. All the drugs for the in vitro study were dissolved in dimethyl sulfoxide (DMSO; **Sigma**-Aldrich).

Organisms. In the present study, we used *C. albicans* ATCC 24433, *C. glabrata* ATCC 90030, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC78, *Candida*

guilliermondii ATCC 9390, *C. neoformans* ATCC 90112,. The strains were obtained from the American Type Culture Collection . We also used clinical isolates of *C. albicans*, provided by N.Amashukeli of Skin and Venerology Desease Clinic and for the investigation of the MICs of "Camelin M". For the in vitro study, strains were cultured on Sabouraud dextrose agar (SDA; Eiken Chemical Co., Ltd., Tokyo, Japan)

In vitro susceptibility testing. The MICs for the test organisms were determined by the broth microdilution method described in NCCLS document M27-A2 (14) for *Candida* spp. and *C. neoformans*. The MICs of "Camelyn M" were defined as the lowest concentration that resulted in slight growth (approximately 90% inhibition) or the absence of growth at 48 h.

Gas chromatography (GC-FID) and gas-chromatography-mass spectrometry (GC-MS)

Gas chromatography (GC-FID) and gas-chromatography-mass spectrometry (GC-MS) was evaluated using the method described by Aviles *et al.* (3). Briefly, gas chromatography appliances used included a Perkin Elmer Auto System equipped with two fused-silica SPB columns (60 m × 0.25 mm i.d.; film thickness 0.25 µm), mounted in parallel in the same oven, with two detectors: FID and Q-Mass 910 (electron ionization 70 eV electron energy, transfer line 220°C). Carrier gas was oxygen and moisture-free helium obtained from a SUPELCO High Capacity Heated Carrier Gas Purifier (Sigma-Aldrich, Milan), provided with an OMI-2 indicating tube, at an average flow rate of 1 mL/min. The oven temperature programme was 60°C for 4 min, then 2°C/min until 180°C was reached, then increased 3°C/min until 250°C. The detector and the injector temperature was 280°C. The volume of injected essential oil or pure substance was 0.1 µL, and the split ratio was 1:50. Two distinct data systems were connected to the GC-FID or GC-MS: Turbochrom and Q-mass Analytical Workstation Software (Perkin-Elmer, Milan) with a NIST/EPA/MSDC Mass Spectral database.

Results

***In vitro* antifungal activity.** Table 1 shows the spectrum of activities of "Camelyn M" and other reference against various fungal strains. "Camelyn M" exhibited potent activities against *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. guilliermondii*, and *C. neoformans*, with MICs ranging from 0.012 µg/ml for all the above listed cases. Table 2 shows the MICs of "Camelyn M" and other reference agents for clinical yeast isolate of *C. albicans*. Table 2 shows the results for *C. albicans* separately for FLC-susceptible (FLC-S) strains (FLC MICs, ≤8 µg/ml) and FLC-susceptible dose-dependent (FLC-S-DD) and FLC-resistant (FLC-R) strains (FLC MICs, ≥16 µg/ml), according to the guidelines of NCCLS document M27-A2 (14). "Camelyn M" exhibited potent activities against *C. albicans* (FLC-S), with MICs at which 90% of isolate are inhibited (MIC_{90}s) of 0.012 µg/ml. "Camelin M" also exhibited potent activities against the FLC-S-DD and FLC-R strains of *C. albicans* (MIC range, 0.012 µg/ml).

TABLE 1. Antifungal spectrum of "Camelyn M"

Organism and strain	MIC (µg/ml)			
	Camelyn M	FLC	ITC	AMB
Candida albicans ATCC 24433	0.012	0.25	0.016	0.12
Candida glabrata ATCC 90030	0.012	4.00	0.12	0.12
Candida tropicalis ATCC 750	0.012	2.00	0.06	0.25
Candida parapsilosis ATCC 22019	0.012	2.00	0.03	0.5
Candida krusei TIMM3378	0.012	32.00	0.06	0.25
Candida guilliermondii ATCC 9390	0.012	2.00	0.03	0.06

TABLE 2. In vitro antifungal activity of "Camelyn M" against clinical isolate of *Candida albicans.*

Organism (clinical isolates) and agent	MIC (μg/ml)	
	50%	90%
Candida albicans		
"Camelyn M"	0.012	0.012
FLC	0.25-8	1.00
ITC	0.016	0.03
AMB	0.12	0.12

Discussion

"Camelyn M" belongs to the natural, antifungal agents with a complex mechanism of action. This preparation comprises myrcene 20.8%, terpinen-4-ol 18.4%, citral 58.8% and protodeltofolyn 2.0%. As it was shown in earlier studies myrcene, citral and terpinen-4-ol are characterized by strong antibacterial and fungistatic properties (13). Earlier investigations of the antifungal properties of essential oil of *Melaleuca alternifolia* Cheel (Tea Tree Oil), containing two critical bioactive constituents terpinen-4-ol and 1,8-cineole have demonstrated that the above compounds have strong fungistatic properties causing metabolic changes in candidas. The fact that "Camelyn M" comprises biologically very active saponin – protodeltofolin is very interesting. This saponin may be met in leaves and rootstocks of *Dioscorea caucasica* and *Dioscorea deltoide*. and condition resistance of these plant with respect to different pathogens. Protodeltofolin is characterized with strong detergent properties and at occurrence in small size organisms cause destruction of the cell membrane. Resulting from the mentioned, it may be presumed that the biological activity of "Camelyn" includes the above mechanisms, which of cause is a subject of deeper investigations.

As it is seen from Tables 1 and 2 "Camelyn M" displays strong in vitro activity with respect to all the test strains in the same concentration -MIC (μg/ml)=0.012. This concentration is more low than all the antibiotics tested by us.

In this study we evaluated the *in vitro* activity of "Camelyn M". This preparation exhibited potent in vitro activities against *C. albicans*, including FLC-resistant strains; *C. glabrata*; *C. guilliermondii*; and *C. neoformans*. *C. albicans* is a key yeast pathogen, and FLC-resistant *C. albicans* has become a clinical problem in AIDS patients with OPC (16, 17).

In conclusion, the results of the present study suggest that "Camelyn M" is a promising compound, in particular, for the treatment of disseminated or mucosal infections induced by *C. albicans*, including FLC-resistant strains.

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Footnotes

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