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**Studying toxicity actions of new pharmacological  
remedy "Camelyn M-3"  
(Report)**

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# ***Investigation of general toxic actions of Ointment "Camelyn M-3"***

**(Acute toxicity, cumulative toxicity, allergic, topical -irritating action)**

## ***Introduction***

Camelyn M-3 is a yellowish - white Ointment with a characteristic smell.

Composition:

Camelyn M -1	5.0 ml
Olive oil	10, 0 ml
White wax	4,0 g
Porpoise alcohol solution	1, 0 ml

"Camelyn M-3" is derived from honey, and is proposed as an antiseptic and wound healing remedy.

Other ingredients of ointment "Camelyn M-3", had been approved by the pharmacology committee at the Ministry of Health of Georgia and been allowed for use as an antiseptic remedy in medicine.

Standard packaging of ointment "Camelyn M-3" is represented by an aluminum tube containing 20g ointment.

According to recommendation, the ointment should be used as a topical application, applied in a thin layer on the affected area of the skin.

Since any proposed new remedy for medical use must be tested for safety, we have studied the general toxicity of Camelyn M-3, concentrating mainly on acute toxicity and cumulative effects, as well as topical reactions (irritation, allergic reactions).

The objective of our investigation was the definition of toxicity of the ointment - Camelyn M-3.

## ***Definition of acute toxicity of ointment "Camelyn M-3"***

Clinical studies of acute toxicity give the opportunity to establish any damaging effects of 'Camelyn M3', both in the case of a single application and when repeated at six-hourly intervals through the day.

***Objectives of study:*** definition of acute toxicity and LD<sub>50</sub> with different dosages of the investigated remedy, description a clinical picture of general resorption and a set of reasons for test-animals deaths.

## **1. Definition of acute and cumulative toxicity of Ointment Camelyn M-3**

### **1.1. Definition of acute toxicity in white mice**

#### **Method.**

Under clinical observation 32 sheared white mice were divided into 4 groups, 8 in each group, with individually identified body weight.

#### **I Group**

Lateral surface skin of mice ( $2 \times 3 = 6 \text{ cm}^2$ ) was treated by ointment "Camelyn M-3", in a dose 250 mg/20g or 4 mg/  $\text{cm}^2$ .

Should be considered that skin's surface of 20, 0 g animal make up - 61  $\text{cm}^2$ .

#### **II. Group.**

Both sides of lateral surface of skin and tail of animals were treated by ointment "Camelyn M-3" in dose 500 mg/20g or 8 mg/  $\text{cm}^2$ .

#### **III Group.**

Both sides of lateral surface skin and tail ( $2 \times 3$  or  $2 \times 3 \text{ cm}^2$ ) of animals were treated by ointment "Camelyn-M3" in dose 1000 mg/20g or 16 mg/  $\text{cm}^2$ .

#### **IV Group.**

Control - animals were treated on lateral surface.

## **Results**

Observation of the animals has given the following picture:

After use of ointment the condition of the animals was checked twice:

#### **- Immediately and within two hours of use of the Ointment -**

Deviations from integrated norm not observed.

#### **-After 24 hours**

All animals willingly for food, remained healthy and active, reflexes and reactions to external irritants were normal and deaths were absent.

#### **Daily observations of test animals over 14 days -**

No deviation from the norm was noted and no deaths of test animals occurred.

Due to the absence of mice loss, an exact estimate of  $\text{LD}_{50}$  is not possible.

Based on the results of experiments on mice, it has been concluded that  $\text{LD}_{50}$  of the investigated remedy exceeds 1, 0 g/20 0 g, which demonstrates that "Camelyn M-3", ointment does not possess resorption action.

## **1.2. Definition of cumulative effects of Ointment "Camelyn M-3" in mice**

### **Method.**

Under clinical observation 32 sheared white mice were divided into 4 groups, 8 in each group, with individually identified body weight.

### **I Group.**

Lateral surface skin of mice ( $2 \times 3 = 6 \text{ cm}^2$ ) was treated with ointment "Camelyn M-3", in a dose 250 mg/20g or 4 mg/  $\text{cm}^2$ .

Should be considered that skin surface of 20, 0 g animal, make up 61  $\text{cm}^2$ .

### **II Group.**

Both sides of lateral surface skin and tail of animals were treated by ointment "Camelyn M-3" in dose 500 mg/20g or 8 mg/  $\text{cm}^2$ .

### **III Group.**

Both sides of lateral surface skin and tail ( $2 \times 3$  or  $2 \times 3 \text{ cm}^2$ ) of animals were treated by ointment "Camelyn M-3" in dose 1000 mg/20g or 16 mg/  $\text{cm}^2$

### **IV Group. Control animals.**

Both sides of lateral surface skin, on the back and tail of animals, were treated by 1000 mg/20g of propolis spirituous solution and olive oil in ratio 1:1.

The mice were treated with the above mentioned solution daily for 3 days.

## **Results**

Observation of the animals has shown the following picture:

After use of the ointment, the condition of the animals was checked twice:

### **- Immediately and within two hours of use of ointment:**

Deviations from integrated norms were not observed.

### **- After 24 hours**

All animals willingly for food, remained healthy and active, reflexes and reactions to external irritants were normal and deaths were absent

### **The second application of ointment showed:**

-After 48 hours from the first using of ointment,

all animals were alive, active, unconditioned reflexes and reactions to external irritants normal. Animals willingly for food..

### **72 hours from the first application of remedy:**

No deaths were observed.

All animals remained active, reflections and reactions adequate, willingly for food

**Daily observations of the animals over 14 days.**

No deviation from the norm was revealed, and no deaths were observed.

Due to absence of mouse loss, an exact estimate of LD<sub>50</sub> is not possible.

Based on the results of experiments on mice, it has been concluded that the investigated remedy Ointment "Camelyn M-3" does not possess resorption action.

**1.3. Pathology- morphological research on euthanized mice, after definition of acute toxicity and cumulative effect of investigated remedy****External examination before euthanasia:**

Animals were mobile, coats pure and smooth, the skin on the back pink and without defects; lesions and haemorrhages absent, ears and tails pink without haemorrhages, reflexes adequate.

**Description report (after euthanasia) on incision**

Internally organs were located correctly, mucous cover pink; velvety, damp, transparent, free from defects and haemorrhaging; well-developed fatty tissue, cardiac muscle elastic, lungs pink, light; Thymus was not increased, haemorrhages not present, liver, kidneys unswollen, surface smooth, haemorrhages not present. Gall-bladder full; no haemorrhaging of adrenal glands.

Spleen unswollen, surface smooth, light, haemorrhages not present, urinary bladder full, urine transparent.

The organs of the gastrointestinal tract were unchanged; no haemorrhages on the mucus membrane of the mouth and oesophagus, the tongue clear and without haemorrhages.

**Resume**

Toxicological Pathology- morphological research of the mice has shown that toxicity and cumulative effect of Ointment "Camelyn M3" is absent.

**1.4. Definition of acute toxicity of ointment "Camelyn M-3" on rats****Method**

Under clinical observation were 32 sheared white rats, divided into 4 groups, 8 in each group, with individually identified body weight.

**I Group.**

Lateral surface skin of rats ( $2 \times 3 = 6 \text{ cm}^2$ ) was treated with ointment "Camelyn M-3", in a dose 5 g / 200g or 14,8 mg/  $\text{cm}^2$ .

Should be considered that skin surface of rats with 200 g body weight makes up - 304 sm<sup>2</sup>.

***II Group.***

Both sides of lateral surface skin and tail of animals were treated with ointment "Camelyn M-3" in dose 7, 5 g /200 g or 22.2 mg/ sm<sup>2</sup>.

***III Group.***

Both sides of lateral surface skin and tail (2x3 and 2x3 sm<sup>2</sup>) of animals were treated with ointment "Camelyn M-3" in dose 10 g / 200g or 37 mg/ sm<sup>2</sup>.

***IV Group. Control-animals.***

Both sides of lateral surface skin, back and tail (2x3 and 2x3 sm<sup>2</sup>) of animals were treated with 10g/200g mass of propolis spirituuous solution and olive oil in ratio 1:1.

***Results***

Observation of the animals has shown the following picture:

**- Immediately and within two hours of applying the ointment:**

No deviations from integrated norm were observed.

**-After 24 hours**

All animals willingly for food, remained healthy and active, reflexes and reactions to external irritants were normal and deaths were absent.

**Daily observations of the animals over 14 days.**

No deviations from the norm were recorded and no dead animals observed.

Due to absence of test-animal loss, exact estimate of LD<sub>50</sub> is not possible.

Based on the results of experiments on rats, it has been concluded that LD<sub>50</sub> of the investigated remedy exceeds 10 g/200 g, thus demonstrating that "Camelyn M-3" ointment does not possess resorption action.

***1.5. Definition of cumulative effect of remedy  
Camelyn M-3 on rats***

**Method**

Under clinical observation 32 sheared white rats were divided into 4 groups, 8 in each group, with individually identified body weight.

***I Group.***

Lateral surface skin of rats (2x3=6 sm<sup>2</sup>) was treated with ointment "Camelyn M-3" in a dose 5g/200g or 14,8 mg/ sm<sup>2</sup>.

Should be considered that skin surface of 200 g rats constitutes - 304 cm<sup>2</sup>.

**II Group.**

Both sides of lateral surface skin and tail of animals were treated with ointment "Camelyn M-3" in dose 7, 5 g / 200g of mass or 22, 2 mg/ cm<sup>2</sup>.

**III Group.**

Both sides of lateral surface skin and tail (2x3 and 2x3 cm<sup>2</sup>) of animals were treated with ointment "Camelyn M-3" in dose 10 g/200g of mass or 37 mg/ cm<sup>2</sup>.

**IV. Group.**

Both sides of lateral surface skin, on the back and tail of rats, were treated with 15 mg/200 g mass of propolis spirituous solution and olive oil in ratio 1:1.

The animals were treated with the above mentioned solution daily for three days.

**Results**

Observation of the animals has shown the following picture:

After administration of the ointment, the condition of the test-animals was checked twice:

**- Immediately and within 30 minutes of application of the ointment:**

No deviations from integrated norms were observed.

**-After 24 hours**

All animals were healthy, active, unconditioned reflexes and reactions to external irritants were normal, animals willingly for food. No dead animals were observed.

**The second application of the ointment showed:**

**48 hours from the first application of ointment:**

- All animals were alive, active, unconditioned reflexes, reactions to external irritants were normal, willingly for food. No dead animals were observed.

**The third application of the ointment showed:**

**72 hours from the first application of ointment**

All animals were active, reflexes and reactions adequate, no dead animals were observed, willingly for food.

**After Daily observations of the animal over 14 days.**

All animals were active, willingly for food. Reflexes and reactions to external irritants were normal.

Due to absence of test-animal loss, exact estimate of LD<sub>50</sub> is not possible.

Based on result of experiments it has been concluded that Ointment "Camelyn M-3" does not possess cumulative and resorption actions.

## ***6. Pathology - morphological research of the euthanized rats, after definition of acute toxicity and cumulative effect for Ointment "Camelyn M-3" (investigated remedy)***

### **External examination before Euthanisation**

Animals were mobile, coats pure and smooth, skin on back pink, without defects, haemorrhages not present, Ears, tail pink, without haemorrhages. Reflexes normal, adequate.

### **Description report (after euthanisation) on incision.**

Internal organs located correctly, mucus cover pink, velvety, damp, transparent, free from defects and haemorrhage, well-developed fatty tissue, cardiac muscle elastic, lungs pink, light; Thymus unswollen, haemorrhages not present; liver, kidneys unswollen, surface smooth, haemorrhages not present.

Gall-bladder full, no haemorrhages present in adrenal glands, spleen unswollen, surface smooth, light, haemorrhages not present; urinary bladder full, urine transparent.

Gastrointestinal tract organs unchanged, no haemorrhages present on mucus membrane of mouth and oesophagus, tongue clear and without haemorrhaging.

### **Resume**

Toxicological and Pathology – morphological research on rats has shown that

Camelyn M3 ointment does not possess acute toxicity or cumulative effects.

## ***1. Definition of Allergic action of Ointment" Camelyn M-3" on rats.***

### **Method.**

For the clinical study of allergic effect on rats was used. a skin application.

20 white rats with body weight 18 - 20 g were used in clinical trials, 10 to receive the treatment and 10 for control.

The investigated animals were sheared (size 2, 0 x 2, 0 sm) on a lateral surface, close to the centre of the body, and the cleaned surface of the skin treated with (investigated remedy) 0,5g ointment. The skin application was carried out daily during one month.

A thin layer of the preparation was applied to the full surface of the skin using a glass spatula.

Control animals were treated with 0.5 r propolis spirituous solution with olive oil in the ratio 1-1.

Results were examined after 30 applications. Size of skin's fold was measured by shtangencircul in both treated and control animals.

### **Results**

After treatment with ointment the skin remained smooth, pure; haemorrhages, rashes, lesions and hypostases were not present.

The average size of a skin fold in treated animals was 1, 16 mm;  
In control animals - 1, 14 mm.

Treated skin surface colour and temperature did not differ from surrounding tissue, nor from tissue of animals from control group.

The rats were euthanized by decapitation.

### **Description report on incision**

Internal organs located correctly, tissue pink, fatty tissue well developed, pleura, peritoneum smooth, haemorrhages not present, organs not increased in size, moist, light.

### **Resume**

On the basis of experimental data it is possible to reach the following conclusion:  
The investigated remedy - Ointment "Camelyn M-3" - does not cause allergic reactions.

### **3. Topical -irritating effect of Remedy Camelyn M-3**

During study of acute toxicity and accumulative action on mice and rats insignificant topical-irritating effects were noted after 30 applications.

Results were examined under magnification.

After treatment the skin was smooth, clear, haemorrhages, rashes, lesions and hypostases were not present.

### **Resume**

On the basis of experimental data it is possible to conclude that the investigated remedy Ointment "Camelyn M-3" does not possess topical - irritating action.

### **Conclusion**

Experimental studies with skin application on mice and rats have shown following:

Ointment "Camelyn M-3" does not possess resorption effects.

The maximum permissible dose for rats and mice has not developed acute and cumulative toxicity.

**LD<sub>50</sub>** ointment - for mice exceeds 1g/20 g body weight and for rats 10g/200g body weight.

Experimental studies on mice have shown that ointment "Camelyn M-3" possesses no topical-irritating effects.

Experimental studies on rats has shown, that ointment "Camelyn M-3" possesses no allergenic effects.