

# Clinical Studies

1. Anadolu university, faculty of pharmacy, department of pharmaceutical technology cosmetology department, complex B herbal extract 26470 eskisehir, Complex B herbal extract.
2. Turkish republic istanbul university istanbul medical faculty dermatology department, investigation of efficacy, safety and permanence of bioxet body hair inhibitor creme.
3. Anadolu university, faculty of pharmacy, department pharmaceutical technologies cosmetology department 26470 eskisehir, Bioxet body hair inhibitor and skin care crème.
4. Anadolu university, faculty of pharmacy, department pharmaceutical technologies cosmetology department 26470 eskisehir, Physical tests.

## 1. Complex B Herbal Extract

### Physical tests

On the freshly prepared product (complex B extract) microbiological and rheological analyses were performed; its pH value was measured and sample was physically examined.

- It is seen as a homogenous solution and no phase separation was observed.
- Herbal extract is off-yellow.
- pH value of complex B was 4.92. This pH value is suitable for applications on the skin. Viscosity of complex B was 716 mPas sec. It is suitable for applications on the skin.
- Contaminants obtained from microbiological analysis are non-pathogenic and very common ones.

### Stability tests storage conditions

Product (complex B) samples were stored under conditions; 60% relative humidity and  $4\text{°C}\pm 1\text{°C}$ ,  $25\text{°C}\pm 0.1\text{°C}$ ,  $40\text{°C}\pm 0.1\text{°C}$ ,  $-40\text{°C}\pm 0.1\text{°C}$ .

- No changes in appearance or phase separation were observed.
- Off yellow color of the extract became darker in every medium in speeded up stability conditions (fridge, room temperature, stove, acclimatization cabin). This indicates preparation should be filled in oxygen-free mediums and be kept in tightly sealed, colored vessels with small application holes.
- Viscosity changes were observed during storage of complex B. Yet the interval of change was not extensive enough to provide problems for application.
- No microorganism that might cause problems in respect for applications were observed in microbiological analyses.

### **Efficacy tests complex B**

- It had positive effects on skin moisture (13.03%-38.77%) and on sebum (35.66%-546.9%) and it did not cause any dramatic changes in the pH of skin ((-5.51) %-(-18.2) %).
- Hair diameter has thinned in the ratios between 28.3% and 32.3%.
- Hair length has shortened in the ratios between 28.5% and 57%.
- Number of hair has decreased in the ratios between 39.4% and 53%.

Besides the herbal extract was found to have no side effects in the clinic trials conducted as it was observed that it did not mix into systemic bloodstream

## **2. Investigation of efficacy, safety and permanence of BIOXET body hair inhibitor crème.**

The study was conducted on 54 subjects of various ages (44 females and 10 males). The volunteers were held subject to hemogram tests, biochemical serum tests, hormonal tests and other epidemiologic laboratory tests before they were admitted to the study. Subjects with no pathologies as determined in the clinical and laboratory examinations and using no medications were subjected to dermatological examinations and locations, density, thickness, rigidity and color of their body hairs were determined and their body hairs were pictured and examined with a special microscope. Crème was applied to the subjects and their skins were tested (in respect for allergies and irritation).

Undesired body hair of the subjects with no allergies or irritations was depilated with a suitable method and crème was applied to them by massage once a day to the predetermined areas. The volunteers were subjected to regular clinical and laboratory examinations and were followed up for a period of eight months.

Results taken at the end of eight months:

27.7% retard in growth of body hair 50% decrease in the amount of body hair 25.9% thinning and softening of body hair 7.4% color change in body hair.

Besides the subjects with positive results were followed up for a period of an additional eight months during which they did not use BIOXET crème. No changes were observed in the thinned; less dense or discolored body hair at the end of the second eight months.

As a result it was concluded that BIOXET crème is an herbal product that could be used safely by females and males on all parts of the body at any seasons for the purpose of minimizing undesired body hair. It is a product, which can provide the desired effect when used as instructed, with minimal and temporary local side effects and with no systemic side effects and non-changing the normal characteristics of skin.

### **Cytotoxicity tests. Tests based on mitochondrial activity and measurement of MTT**

While significant amount of cytotoxicity could not be observed with a concentration of 200 µg/ml, it was determined that the only dose that demonstrated cytotoxic effect for cell metabolism for each of the 4 days was 1000 µg/ml.

### **Tests based on lysosomal activity and neutral measurement**

RED UP-take (NR). In the first day lysosomal enzyme inhibition was not observed with concentrations of 40, 200 or 1000 µg/ml.

In other days and with other concentrations an increase was observed in the lysosomal activity of enzymes when compared with control subjects.

An aqueous extract is present in the crème in the ratio of 70% and if 1.5% of this is solid extract (based on the data obtained from lyophilization) then 1.106 gram extract is present in 100 grams of crème. According to the data that we have obtained; as the cells applied with crème reproduce in two dimensions, data may be given in cm<sup>2</sup>; for a skin of 1 cm<sup>2</sup>.

The maximum crème amount that can be applied will be equivalent to an extract of 40 mg which gives the amount of crème equal to 3.6 grams. Maximum 3.6 grams crème may be applied to 1 cm<sup>2</sup> skin everyday. Biological barrier effects in cytotoxicity values on fibroblasts can not be determined with these tests. Crème applications up to 720 mg/cm<sup>2</sup> x application were safe in respect for MTT and NR results based on the 8 µg/ml data.

Besides IC50 was not reached for no dose that was applied.

### **3. BIOXET body hair inhibitor and skin care crème**

In the study performed on volunteers of ages between 39 ±9 consisting of 20 subjects (13 females and 7 males) for a period of 3 months the following results were obtained:

- BIOXET crème demonstrated 3% increase in the moisture level of the skin. When BIOXET crème applied, the areas were compared with other areas statistically, and a significant difference was observed.
- Percentage increase in the skin sebum of the area applied with BIOXET crème was found to be four times higher in comparison to the other areas.
- pH value of skin applied with BIOXET crème was determined as 5.22. Other areas were more acidic and a statistical difference was observed between the areas.
- As a result of the melanin and erythema measurements it was determined that BIOXET crème is safe for dermatological use.
- It was observed that BIOXET crème ensures a significant amount of decrease in the number and diameter of body hair.

As a result it was determined that BIOXET crème is safe for dermatological purposes and is a physico-chemically stable product ensuring a significant decrease in the number and diameter of body hair and having positive effects on skin moisture and sebum when applied for a period of 3 months.

#### 4. Physical test

On the freshly prepared BIOXET crème microbiological and rheological analyses were performed; its pH value was measured and crème was physically examined.

Results:

- BIOXET crème is seen as a homogenous crème and no phase separation was observed.
- It is off-yellow resulting from herbal extract.
- Viscosity of BIOXET crème was 2825 mPas sec and it is suitable for applications to the skin.
- Dew dimensions of BIOXET crème is 2.698  $\mu\text{m}$  and is suitable for applications to the skin.
- No microorganisms that would cause problems in respect for applications of the crème were observed in the microbiological analyses.

#### Stability tests storing conditions

BIOXET crème samples were stored under conditions; 60% relative humidity and 4oC, 25oC, 40oC and -40oC for a period of 3 months.

- No changes in appearance or phase separation were observed.
- Off yellow color of the BIOXET crème became darker in every medium in speeded up stability conditions (fridge, room temperature, stove, acclimatization cabin). This situation indicates that crème should be filled in oxygen-free mediums and be kept in tightly sealed, colored vessels with small application holes.
- No change in pH value was observed.
- Viscosity changes were observed during storage of BIOXET crème. Yet the interval of change was not extensive enough to provide problems for application.
- Dew dimensions of BIOXET crème is between 1-10  $\mu\text{m}$ , which is required for a cosmetics preparation.
- No microorganism that might cause problems in respect for applications were observed in microbiological analyses.

#### Efficacy tests BIOXET crème

Body hair inhibitor BIOXET crème had positive effects on moisture of the skin (19.24%-36.23%) and on sebum (37.94%-1577.0%) and it did not cause any dramatic changes in the pH of skin ((-1.5) %- (-22.1) %). 1.41% increase in the forearm erythema value is in the acceptable limits. That is the use of product is safe. A significant decrease was obtained in the length of body hair (8.68%-28.10%) and diameter of body hair (17.68%-26.60%) for body hair in legs and arms.