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RESEARCH ARTICLE
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FORMULATION AND TESTING OF HERBAL UNDER EYE SERUM PREPARED FROM *CITRULLUS LANATUS* FRUIT EXTRACT

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Abstract
Background: Dark circles, wrinkles and puffing eyes has become a peril for today's generation. These problems exert a significant psychological impact on a person's quality of life, especially among young girls. It may be due to the extensive use of alcohols, allergies, age factors, genetics, nutritional deficiencies and imbalanced diet, lacking of vitamins and minerals like vitamin C, iron or zinc, excessive use of computer and mobile screens, stress, insomnia or sleep disturbances due to hectic life styles.

Objective: The present project was designed to resolve the problem of dark circles and other under eye skin problems with a suitable cost effective formulation with least adverse effects.

Methodology: This formulation of a serum to be applied under eye portion was designed and tested after continuous experimentation using standard reported methods. The quality control tests were performed for samples along with acute dermal toxicity test.. Phase 1 Clinical Trials were conducted on 25 participants aged between 18-55 years after consent and ethical approval. Finally a questionnaire was filled by the participants at the end of study period of two months.

Results: Most of the participants of study exhibited high satisfaction levels with treatment outcomes. No safety concerns were noted. The study revealed that *Citrullus lanatus* herbal under eye serum is highly significant for treating dark circles, puffy eyes and wrinkles.

Conclusion: The research study could lead to safe and cost effective herbal serum to manage dark circles. However further studies required at molecular level to find out mode of action.

Key Words: Natural cosmetics, Dark circles, *Citrullus lanatus*, under eye serum.

Introduction

Dark circles, wrinkles and puffing eyes has become a peril for today's generation. It may be due to the extensive use of mobile phones, lap tops and other electronic devices. Commercially available cosmetics are full of synthetic chemicals like parabens. The concern with these chemicals is that scientific studies suggest that parabens can absorbed quickly by skin causes allergic reactions, which results in severe adverse effects (Global IVF, 2017). The menace of growing problems of skin is increasing day by day due to the environmental changes, pollution and modern life style (NCATS, 2021). Organic cosmeceutical is a newer term used for cosmetic products containing natural ingredients which can solve the problem of skin.

The present project was designed to resolve the problem of dark circles and other under eye skin problems with a suitable cost effective formulation with least adverse effects. The formulation of a serum to be applied under eye portion was designed and tested after continuous experimentation on pilot scale, which was then tested for quality control to meet the recommended standards. The test formulation was applied to research animals for acute dermal irritant test (OECD, 2017) and after having the positive result in animal testing, it was tested on 25 human participants of both genders of ages between 18-55 suffering from dark circles, puffy eyes and light wrinkles below the eyes. Standard protocol was adopted i.e. explaining the study design, ingredients of the products, filling the consent form and approval of the study by IERB Reference No IBC KU 218/2021dated 1st September 2021 (Iasonos, and O'Quigley, 2021). A complete medical history and physical examination was also encouraged prior treating the aesthetic component (Roberts, 2014). Topical agents that improve blood circulation and/or reduce melanin in the periorbital region are most suitable and convenient as first-line therapy (Chandrashekar BS, Soumya, 2022).

Materials and Methods

Collection and Identification of Plant Material

The plant material was collected from the local market of Karachi and identified by Prof. Dr. M. Mohtasheem ul Hasan, Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical sciences, University of Karachi. The sample was submitted in Department Herbarium and a voucher specimen No. CLF-05-20 was issued for the sample.

Preparation of Extract

The plant material i.e. Fruit of *Citrullus lanatus* was washed and cut into pieces. The pulp was pressed to obtain the juice. Seeds were separated from the juice and the juice was evaporated for removal of excess water through lyophilization method and gel form extract was obtained which were used for further studies.

Formulation of Citrullus lanatus Under Eye Serum

Citrullus lanatus extract was taken with other herbal ingredients used for dark circles and wrinkles and mixed thoroughly until formation of uniform serum. The active ingredient was added in 10% to other herbal material comprising of rose water and *Aloe vera* gel with a little portion of almond oil. No synthetic preservative was added (Figure 1).

Storage condition: Protect from heat and light placed in refrigerator.

Quality Control Tests

The quality control tests were performed for test sample i.e. *Citrullus lanatus* Organic Under Eye serum according to the standard reported method for herbal formulations. All tests were performed at 25^{o}C and were repeated thrice for affirmation. The tested parameters were appearance, homogeneity, pH (digital pH meter JENCO Model VisionPlus pH6175) and viscosity (Brookfield viscometer) according to the reported method with paddle No. 2 at a speed of 30 rpm (Badwaik et al 2022, Mei X Chen et al, 2016).

Acute Dermal Irritant Test

Acute Dermal Toxicity Test was performed on healthy adult rats of both genders weighing between 200-300g. The procedure adopted was taken from OECD Guidelines as exhibited in figure 2 (OECD, 2017). The dorsal side of the animals was shaved one before performing the test. The test product was applied at a dose of 0.2ml uniformly covering the shaved area for 24 hours and the skin was covered with porous gauze dressing. At first one male and one female animal were used for each product. As there was no sign of skin toxicity like itching, redness, inflammation, death so the next step was continued with a dose of 0.4ml on two animals and 0.6ml on further two animals. The tested animals were further kept in observation for 14 days to confirm the result. The animal observation period was 0, 0.5, 6, 12, 18 and 24 hours after application on first day and once daily up to 3 days.

Study procedures and data collection

Application of under-eye serum on human: All subjects were instructed to apply the under-eye serum twice a day for three months. They were allowed to only wash and cleanse their faces and under the eyes but they were not allowed to use any day and night creams or cosmetics under the eyes but allowed to apply on face. They were advised to avoid excessive exposure to sunlight and if necessary covered their eyes with sunglasses.

Study visits

The study participants completed four visits: at baseline (Visit 1), one month (Visit 2), two months (Visit 3), and three months (Visit 4).

Phase 1 Clinical Trials

Phase 1 Clinical Trials were conducted after clearance of Acute Dermal Irritant Test which showed that the product was safe for human use to be applied topically. Before starting clinical studies a standard Consent Form was designed for this research project. As the product is organic cosmeceutical so there was least chance of adverse effects. The study was approved from the Institutional Bioethical Committee bearing approval No. IBC KU 218/2021.

25 participants of both genders ages between 18-55 years were selected for the study and consent was taken and form was duly filled and signed after explaining well the research study. The participants were provided the sample and explained the method of application and were contacted monthly for the feedback. Finally a questionnaire (Figure 3) was filled by the participants at the end of their study period.

Statistical Analysis

The data was statistically analyzed by SPSS using dependent and independent variables using Chi square Tests. The analysis is represented in Table 3-6 showing confidence level 95% and p value $\leq$ 0.05

Result and Discussion

In present investigation, the herbal under eye serum is formulated using *Citrullus lanatus* pulp as active ingredient. The serum was standardized according to reported protocol using various Quality Control Tests and was subjected to albino rats for Acute Dermal Irritant Test to determine the safety of the product for human skin. The formulation was evaluated through Phase I clinical trials after approval of research study through IERB.

Citrullus lanatus is a plant widely grown in Pakistan. The fruit of this plant is nutritive and full of important phytoconstituents like alkaloids, sterols, triterpenes, vitamins and minerals. The important caretenoids identified include lycopene, beta carotene, phytofluene, phytoene, lutein and neurosporene. The pharmacological properties like antimicrobial, antioxidant, analgesic, anti-inflammatory, antiulcer, antidiabetic and hepatoprotective activities were also discussed. The high amount of lycopene i.e. 45.316µg/g provides important medicinal properties like anticancer and anti-inflammatory activities. This fruit also possess citrulline in high amounts which is a precursor of arginine (Erhirhie and Ekene, 2013; Nadeem et al, 2022).

After inducing acute dermal irritant test, there was no sign of skin toxicity like itching, redness, inflammation at all doses and animals were healthy, active and alert and no mortality was reported during the study.

Quality control tests were performed according to the method described above and the results are presented in table 1. The results shows that the formulation is stable for two months and at the end of third months there was no change in appearance while slight change in pH and viscosity. Further research is required to fix these issues.

In Phase 1 Clinical Trials as expressed in Table 2 and further explained through pie charts of Figure 4, nine male and sixteen female participants aged between 18-55 years were included among which 11 were students of different institutes, 5 were self-employed, 4 were employed in private organizations and 5 were unemployed. 8 of them were facing problems of dark circles and puffing eyes, 2 were having only dark circles and 5 were complaining about wrinkles around the eyes. Period of problem was 0.5, 1 and 2 years for 8, 9 and 8 participants respectively. 13 were relieved in 15 days, 9 required one month for recovery and 3 took two months to recover. A large no of participants rated the test product as appealing and excellent while few rated average and satisfactory.

Conclusion

The study revealed that *Citrullus lanatus* herbal under eye serum is highly significant for treating dark circles, puffing eyes and wrinkles. The research study could lead to safe and cost effective herbal serum to manage dark circles. However further studies required at molecular level to find out mode of action.

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Table 1: QC Tests for *Citrullus lanatus* Organic Under Eye Serum

Parameters	**Initial**	**1st Month**	**2nd Month**	**3rd Month**
Appearance	Appealing	Appealing	Appealing	Appealing
Color	Reddish Orange	Reddish Orange	Reddish Orange	Reddish Orange
Odor	Fruity	Fruity	Fruity	Fruity
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
pH	5.203±0.005774	5.187±0.005774	5.133±0.057735	5.06±0.01
Viscosity(mPa.s**)**	24.000±0	23.333±0.57735	22.333±0.57735	21.667±0.57735

Table 2: Clinical Trial Data

S. No	**Gender**	**Age**	**Profession**	**Problem**	**Type of problem**	**Recovery time**	**Product appearance**	**Rate the product**
1	M	28	Self employed	0.5	Dark circles, puffing eyes	1	Appealing	Excellent
2	F	22	Employed	1	Dark circles, puffing eyes	0.5	Appealing	Excellent
3	F	20	Student	1	Dark circles, puffing eyes	1	Appealing	Satisfactory
4	F	21	Student	1	Dark circles, puffing eyes	1	Appealing	Excellent
5	F	20	Student	2	Dark circles, puffing eyes	1	Appealing	Excellent
6	F	19	Student	1	Dark circles, puffing eyes	0.5	Average	Very Good
7	F	20	Student	1	Dark circles, puffing eyes	1	Appealing	Very Good
8	F	24	Self Employed	1	Dark circles, puffing eyes	0.5	Appealing	Excellent
9	F	20	Student	0.5	Dark circles, puffing eyes	0.5	Appealing	Excellent
10	F	21	Self employed	2	Dark circles, puffing eyes	0.5	Appealing	Excellent
11	M	20	Student	0.5	Dark circles, puffing eyes	0.5	Appealing	Excellent
12	F	35	Employed	1	Dark circles, puffing eyes	1	Average	Satisfactory
13	M	22	Student	0.5	Dark circles	0.5	Appealing	Excellent
14	M	55	Unemployed	2	Dark circles, puffing eyes	1	Average	Satisfactory
15	M	22	Student	0.5	Dark circles, puffing eyes	0.5	Appealing	Excellent
16	M	23	Student	0.5	Dark circles, puffing eyes	0.5	Appealing	Excellent
17	M	45	Employed	0.5	Dark circles	0.5	Appealing	Excellent
18	F	55	House Wife	2	Wrinkles	0.5	Average	Good
19	F	45	House wife	2	Wrinkles	1	Average	Satisfactory
20	M	56	Unemployed	2	Wrinkles	2	Average	Satisfactory
21	F	51	House wife	1	Wrinkles	1	Appealing	Excellent
22	M	20	Student	0.5	Dark circles, Puffing eyes	0.5	Appealing	Excellent
23	F	37	Employed	1	Dark circles, puffing eyes	0.5	Average	Good
24	F	47	Self Employed	2	Dark circles, puffing eyes	2	Average	Good
25	F	47	Self Employed	2	Wrinkles	2	Average	Excellent

Note: Age is mentioned in years; Problem existing in years; Recovery Time in months

Table 3: Statistical Calculation by SPSS showing descriptive stats

Descriptive Stats					
	N	**Minimum**	**Maximum**	**Mean**	**Std. Dev.**
Age	25	19	56	31.80	13.772
Problem Duration in years	24	50	2.00	1.1250	0.61237
Recovery Time in Months	25	50	2.00	0.8600	0.48990
Valid N	24				

Table 4: Statistical Calculation by SPSS for Gender variable

	Gender				
		Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid	**M**	9	36.0	36.0	36.0
	F	16	64.0	64.0	
	Total	25	100.0	100.0	100

Table 5: Statistical Calculation by SPSS Rating by Gender as variable

Rating	**Gender**		**Total**
	Male	**Female**	

Excellent	6	8	14
Very Good	1	2	3
Good	0	3	3
Satisfactory	2	3	5
Total	9	16	25

Table 6: Chi Square Test

	Value	**df**	**Asymptotic Significance (2-sided)**
Pearson Chi Square	2.017*	3	0.569
Likelihood Ratio	3.000	3	0.392
Linear by linear Association	1.524	1	0,217
N of valid cases	25		

a. 6 cells (75%) have expected count less than 5. The minimum expected count is 1.08

Figure 1: Test Product

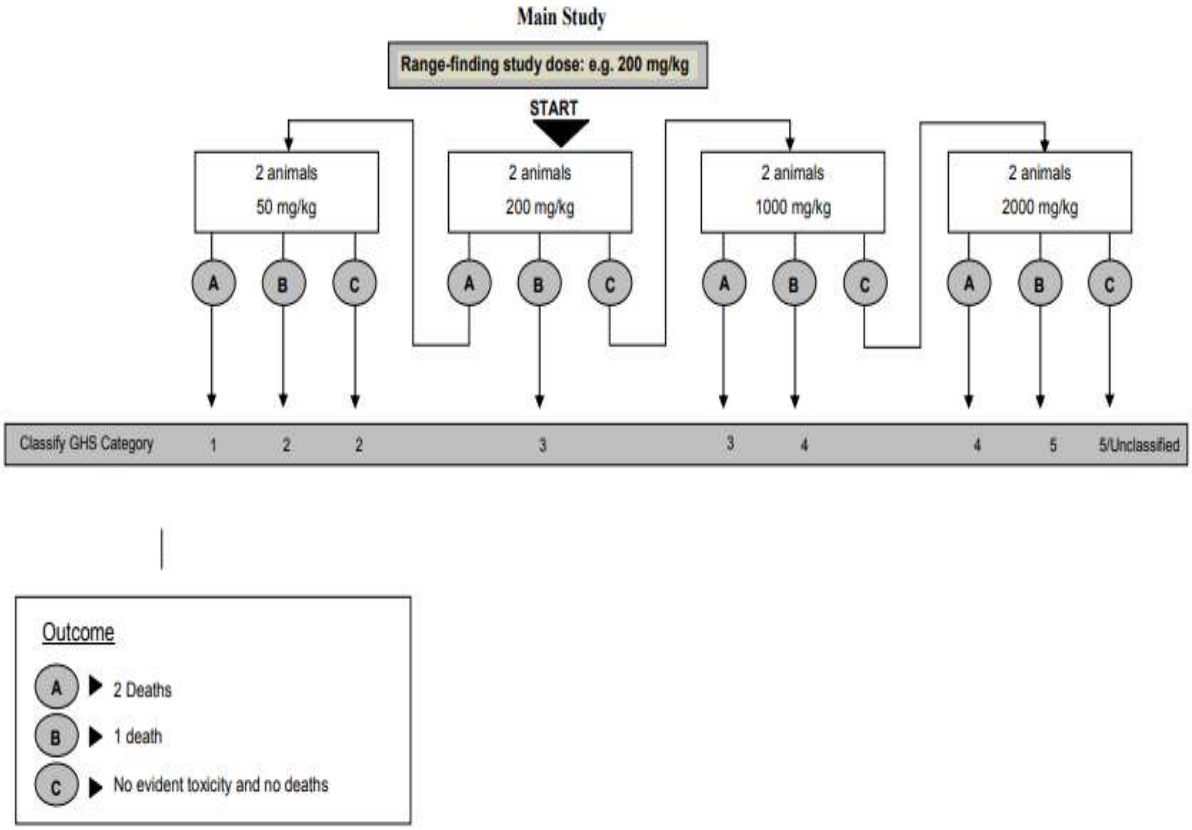

Figure 2: Flow Chart for Testing Procedure

Gender Age Profession

Figure 4: Clinical Trials for *Citrullus lanatus* Organic Under Eye Serum