Research Article

FORMULATION AND EVALUATION OF HERBAL ANTI-AGEING CREAM CONTAINING POMEGRANATE SEED OIL

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ABSTRACT:

Skin is the most exposed organ of the body, is crucial for establishing social bonds and defending against environmental harm. Dermatologist and cosmetic concerns about skin ageing are growing quickly as our society's ageing population grows. Cell death is brought on by oxidative stress, which also causes protein denaturation, lipid per oxidation, and intracellular DNA damage. This study evaluated the anti- wrinkle effects of pomegranate seed oil using a photo aged hairless rat model. The research article also discussed the evaluation methods used to test the efficacy of pomegranate seed oil-based anti-ageing creams. The results of the studies suggest that pomegranate seed oil-based anti-ageing creams can be effective in reducing the signs of ageing. Data shows that the anti-ageing cream (anti wrinkle) treatment greatly improved skin condition of rat suffering from UVB-induced photo ageing , based on the parameters including the skin erythema index, wrinkle area measurement, allergic response, skin texture. The goal of the research was to develop anti ageing cream for decreased ageing, photo ageing, and provide moisturizing, nourishing, whitening, and treating various skin diseases.

Keywords: Cosmetics, pomegranate seeds oil, extrinsic skin ageing, photo ageing, rat models.

INTRODUCTION:

The face is exposed to sunlight continuously from birth, causing the skin to progressively accumulate damage that causes obvious indications of aging to occur by marking specific areas of the skin or recurring facial expressions. Continuous UV exposure can also cause various changes that are classified as photo-induced damages, including vascular homogeneities and pigmentation loss, elasticity loss in the skin, and texture deterioration (elastosis, hyperkeratosis, and yellowing). [1]

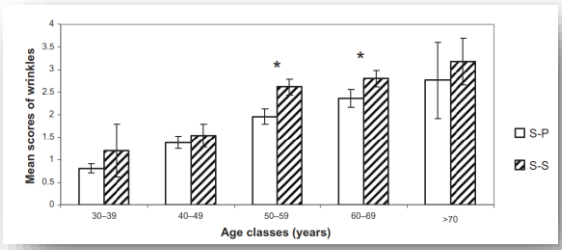
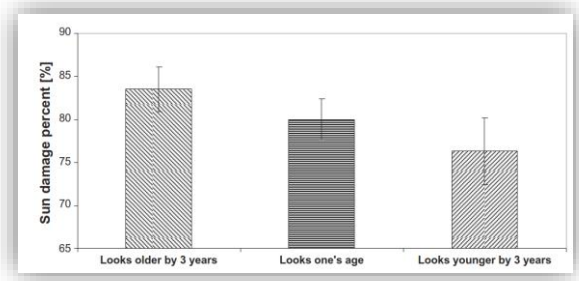


Fig- 1
S-P means Sun Phobic and S-P means Sun Seeking



From the Above figure-1 it shows that In Australian women have the higher number of aging as compared to USA, UK and Canada. The reason of aging is due to the UV exposure, it happens due to geographical location of the Australia is southern hemisphere and northern tip of Australia is closer to equator region due to which it is higher prone to direct exposure to sun and the greatest differences were seen between Australian and US women, the Australian and US women, the Australian women reporting some signs of advanced ageing approximately 20 years earlier than those from the USA.[2]

One of the main indicators of physical aging is aging of the skin. The aging process of the skin is influenced by hormone fluctuations, metabolic processes, genetics, and environmental exposure. Therefore, it is thought that the main contributors to skin aging are intrinsic and extrinsic damage. The

skin uses a range of natural antioxidants to guard against damage caused by free radicals.[3]

The word "cosmetics" comes from the Greek word "cosmetics," which meaning "to adorn." In the past, "cosmetics" was the term for any substance intended to enhance and beautify appearance. Humanity has always needed to take care of its skin. Although elegant, smooth skin is a sign of vitality, the flawless appearance is actually the result of an underlying protein called collagen. Collagen acts as a wonderful cushion to maintain skin smooth and stiff while framing an even layer beneath the skin. As we become older, fine wrinkles start to appear. It is an inevitable part of the regular aging process; nevertheless, premature maturing is caused by excessive sun exposure, tobacco smoking, and dehydration. With the right defensive measures, the aging process can be slowed down considerably.[4]

The World Health Organization (WHO) and our nation have been supporting traditional medicine because it is more affordable, widely accessible, and thorough—especially in developing nations. It's also true that 8% of people on the planet receive their primary medical care from medicinal plants. The developed world, along with its citizens, acknowledged the value of traditional medicine and established protocols, guidelines, and treatment standards for ethnomedicine.[5]

One of the first edible fruits, the pomegranate (Punica granatum L.), is a member of the Punicaceae family and has been utilized widely in folk medicine across many civilizations. This fruit is indigenous to Iran, where it is produced extensively along with India, the United States, and other near and far eastern countries. An estimated 1,500,000 tons of this crop are produced globally, with 47% of that amount coming from Iran, which has the largest area under cultivation.[6]

Pomegranates are rich in lipids such as oleic acid, stearic acid, and palmitic acid as well as polyunsaturated fatty acids like linoleic and linolenic acid.[7]

PSO accounts about 12–20% of the overall weight of seeds. About 80% of the contents of seeds are made up of conjugated octadecatrienoic fatty acids, and PSO is thought to be a rich source of these fatty acids, especially punicic acid (PA) (cis9, trans11, cis13 acid), which is the primary fatty acid among them. Pomegranate juice, peels, leaves, and flowers all have strong antioxidant qualities, however the juice, peel, and oil all have mildly estrogenic effects. Pomegranate seeds contain a high amount of conjugated α-linolenic acids (CLn) and ethnomedical indications. Numerous pharmacological effects are associated with pomegranate seed oil (PSO), which has a high concentration of punicic acid (PA), a conjugated isoenolenic acid isomer. Antioxidant, anti-inflammatory, nephroprotective, hepatoprotective, neuroprotective, anti-cancer, strengthening the immune system, improving carbohydrate metabolism, and lowering insulin resistance are some of its primary characteristics.[8]

MATERIALS AND METHODS

MATERIALS

The materials used in this study were pomegranate seeds oil, aloe vera gel, vitamin E, turmeric powder, stearic acid, cetyl alcohol, glycerine, petroleum jelly, methyl paraben, potassium hydroxide, rose water, distilled water. Aloe vera, turmeric, and vitamin E capsule were collected from the local market in Bhopal MP.

Cream formulation

Took the stearic acid, cetyl alcohol, glycerine, petroleum jelly, methyl paraben, and pomegranate seeds oil, in a borosilicate glass breaker at 75˚C and maintain that heating temperatures (oil phase). In other beaker, dissolve potassium hydroxide and methyl paraben in distilled water by maintaining temperatures 75˚C with electric heating mantle. Use a glass rod to stir the solution until the water phase is completely free of all solids. Gently pour the heated aqueous phase into the heated oily phase while continuing to mix. Until it creates a smooth cream, the continues to mix using a glass rod. Add rose water as a fragrance after the cream is fully formed. Put this cream on the slap and, if necessary, add a few drops of distilled water. Then, mix the cream geometrically over the slap to give it a smooth texture and to thoroughly combine all the ingredients. Slap technique or extemporaneous cream preparation is the name of this technique.

Figure 2 Images of formulated cream

S. No	Ingredients	F1	F2	F3	F4	F5	F6
1	PSO	2ml	1ml	3ml	5ml	7ml	8ml
2	Aloe Vera extract	2ml	2ml	2ml	3ml	3ml	2ml
3	Turmeric extract	1ml	1ml	1ml	2ml	2ml	1ml
4	Vitamin E	1ml	1ml	1ml	2ml	2ml	1ml
5	Stearic acid	2gm	2gm	0.5gm	10gm	15gm	12gm
6	Cetyl alcohol	1gm	1gm	1gm	5gm	5gm	3gm
7	Glycerine	1ml	1ml	1gm	5ml	5ml	3ml
8	Petroleum jelly	1gm	1gm	1gm	5gm	5gm	3gm
9	Methyl paraben	0.5mg	0.5gm	0.5gm	2.5gm	2.5gm	2gm
10	Potassium hydroxide	0.5gm	1gm	0.5gm	2.5gm	2.5gm	2gm
11	Distilled water	qs	qs	qs	qs	qs	qs
12	Rose water	qs	qs	qs	qs	qs	qs

Table 1: Formulations of anti-ageing cream

Evaluation parameters of Anti-Ageing Cream:

Various evaluation methods are used to test the efficacy of anti-ageing creams using pomegranate oil. One of the most common methods is the use of in vitro assays. In vitro assays involve testing the cream's efficacy in a laboratory setting. For example, the cream can be tested for its ability to reduce oxidative stress in skin cells using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Additionally, the cream can be tested for its ability to increase collagen production in skin cells using the hydroxyproline assay.[9]

Another evaluation method that can be used is the in vivo method. In vivo studies involve testing the cream's efficacy on human subjects. For example, the cream can be tested for its ability to reduce the appearance of fine lines and wrinkles using digital imaging techniques. Additionally, the cream can be tested for its ability to increase skin hydration levels using skin hydration measurements.

Organoleptic study:[10,11,12]

Organoleptic study for herbal formulation is a sensory analysis that evaluates the sensory characteristics of a product using the human senses of sight, smell, touch. The Organoleptic evaluation of an anti-aging cream of pomegranate oil should be conducted using a scientific approach, including evaluating its appearance, odor, pH, viscosity, Spreadibility, homogeneity, skin feel, removal, irritancy test, and stability. These tests will help ensure that the product is of high quality, safe, and effective for its intended use.[10]

In the case of an anti-aging cream of pomegranate oil, the following Organoleptic evaluation can be conducted in a scientific manner:

Appearance: Evaluate the cream's color, clarity, and texture. The color should be uniform and consistent, while the texture should be smooth and free of lumps or grittiness. Odor: Evaluate the cream's odor for its intensity, character, and stability. The odor should be pleasant and consistent throughout the product's shelf life.

After feel: We measured the amount of emollient, slipperiness, and thickness residue that remained after applying a specific amount of cream.

Removal: It was discovered how quickly the cream could be removed by running tap water over the area where it was applied.

Irritancy test: The Cream was applied to the left hand's specific area, and the time was recorded. Oedema, skin redness, and irritability were monitored up to a 24-hour period at regular intervals.

Grittiness: On a glass slide, a tiny bit of cream was put, and the surface was then illuminated to look for any foreign particles.

Table 2: Organoleptic activity

Sl. No.	Specification	Limits
1	State	Semisolid
2	Color	Yellowish white, white
3	Odor	Characteristic
4	Texture	Smooth

RESULTS AND DISCUSSION

pH: Measure the pH of the cream using a pH meter. In order to calibrate the pH meter, standard buffer solution was used. 0.5g of weighted cream that had been dissolved in 20.0 ml of distilled water and its pH was tested.[11] The pH should be within the acceptable range for the product, typically between 4.5 and 6.5. The formulation's pH was tested at 1, 10, 30, 60 and 90 days after preparation. The results of this research are shown in table number 3.

Table 3: pH observation for various time periods

Temperature	Initial pH	pH after 1 month	pH after 2 month	pH after 3 month
37±1 ˚C	6.80 ± 0.01	6.50 ± 0.04	6.30 ± 0.03	5.80 ± 0.02

Determination of type of cream:

In this experiment, the cream was diluted with either water or oil. Water is the dispersion medium, thus if the cream is diluted with water and remains stable, it is an o/w type cream of cream. However, if the cream is diluted with oil, the cream will break since oil and water are not miscible.[13] In the present study, 2.5 grammes of cream were taken, and the cream was gradually diluted with distilled water.

Figure 3 Dilution test

Viscocity: The viscosity of the cream was measured using a Brookfield viscometer DV-II + Pro (Brookfield engineering laboratories) with cylinder spindle #64, Test samples were collected in 250ml beakers that were clean and dry, and the viscosity of each sample was assessed using the viscometer's regular operating methods. The reading was recording at100% torque. Sample temperatures were 37±1˚C. We read the value in centipoises. The viscosities of formulated anti-ageing creams was found in the range of 3260 to 6499 Cp and it was closure to the standard marketed cream, it indicate that sufficient amount of oil phase and aqueous phase was used during formulation of cream. The results are given in table number 4.

Spreadibility study of cream: A glass device put up in the lab was used to assess the Spreadibility of the cream formulation. The cream emulsion was sandwiched between the two glass slides. A 500 gm mass was then placed on the slide for 1 minute to compress the sample and create a consistent thickness. Extra cream was then scraped off. Spreadibility is calculated using the formula:

Formula of Spreadibility: S = M x L / T

Where, M is the weight that has been put to the top slide, L is the size of the glass slide, and T is the number of seconds.[14] The Spreadibility was found to range about 21 and 30 gm.cm/sec. it was found to be within the Spreadibility range of commercial cream, The cream's formulation made it simple to distribute without creating a lot of friction, the results are given in table number 4.

Microstructure observation: An optical microscope with a 40x magnification was used to analyse the morphology of prepared cream. Before the microscopic examination, a cover slip was placed over the 1gm of cream samples, which had been smeared gently over the slide. The slide was placed on a microscope slide. Photomicrography picture of the emulsion was captured and it was evident that oil globules were present on the continuous water phase, which proves that the anti ageing (Pomegranate seeds oil) cream was properly manufactured.

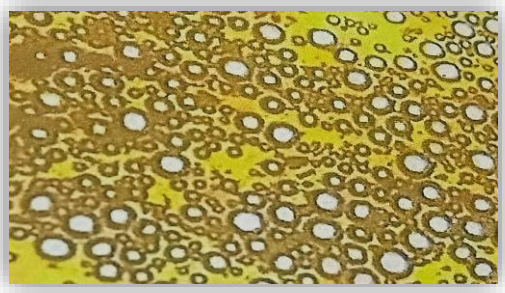


Fig 4: Microscopic structure of cream

Table 4: Cream formulation and their results:

Formulations	pH	Viscosity(cP)	Spread ability(gm*cm/sec)	Sensitivity	Erythema	Phase separation	Wash ability
F1	6.80	7558 ± 1.02	21.6	No irritation	Nil	Not phase separation	Easily washable
F2	6.00	8698 ± 1.00	22.5	No irritation	Nil	Not phase separation	Easily washable
F3	6.50	6839 ± 1.22	20.8	No irritation	Nil	Not phase separation	Easily washable
F4	6.32	5639 ± 2.51	26.6	No irritation	Nil	Not phase separation	Easily washable
F5	6.26	4829 ± 1.33	25	No irritation	Nil	Not phase separation	Easily washable
F6	7.00	4457 ± 1.15	24.1	No irritation	Nil	Not phase separation	Easily washable

STABILITY STUDY OF FORMULATION[15]

Final formulations including active components were kept in plastic containers at 2 to 4˚C, 25˚C and 40˚C. After week 4, all formulations physiochemical characteristics were assessed to determine their physical stability.

The optimised formulation was stored in an airtight container at the specified temperature to gauge the stability of the cream of pomegranate seeds oil. The measurement used for evaluating the stability was determined based on organoleptic properties, pH, and viscosity. At regular intervals of time (0, 1, 2, and 3 months), samples of the pomegranate seeds oil cream were taken and every evaluation parameters was recorded. The results of the stability study are given in the following table number 4.

Table 4: Data of stability study

Temperature	Homogeneity	Phase separation	Simple removal
2-4˚C	Homogeneous	No	Remove quickly
Room temperature	Homogeneous	No	Remove quickly
40˚C	Slightly liquefying	No	Remove quickly

Table 5: Accelerated stability study

Months / Test	Anti-ageing cream		
	Initial month	After two month	After three month
Physical features	Semi solid	Semi solid	Semi solid
Texture	Good	Good	Good
Odour	Characteristic	Characteristic	Characteristic
Thermal stability	Ok	Ok	Ok
Deterioration of goods	Nil	Nil	Nil



IN VIVO EVALUATION OF FORMULATED ANTI-AGEING CREAM:

Both sexes of albino rats (weighing 150–180g) will be used in the studies. The animals must be kept in groups of six in polyacrylic cages measuring 38 by 23 by 10 cm, and they must be kept in a typical laboratory environment with a temperature of 25 °C, a dark/light cycle, and relative humidity between 60 and 70 %. They will consume a pellet diet along with water additives. The animals will become accustomed to the laboratory environment for seven days before the studies begin. Animal research was done at the pharmacology division. Truba institute of Pharmacy, Bhopal (M.P.) with due permission from Institutional Animal Ethical Committee(IAEC approval no. PH/IAEC/TIP/2023), all the research was performed according to the animal ethics committee guidelines for the experimental animals.

Selection of animal model:[16]
There are six animals in each of the four groups into which the animals were divided.

Group 1 will be designated as the standard control.

Group 2 will serve as the negative control. (Ageing induced)

Group-3 will be named as marketed control and ageing will be treated with marketed anti-ageing cream.

Group-4 will be assigned as test groups that will treated with formulated cream

Procedure to develop the ageing: All the animals were anesthetized by chloroform (5ml), after shaving; a hair removal cream was used to completely remove the hair, prepare the ageing induced assembly, and then it will keep under for exposing UV radiation (UVB radiation 320-290nm). This process will take place for 7-10 days, after this process wrinkle develop and the formulated cream and marketed cream was applied regular to all animals for 30 days.

Fig5. Induced ageing

Evaluation parameters:[17]

- Area of skin wrinkles.
- Skin irritation study.
- Photographic comparison.
- Skin texture.
- Allergic response.

1. Area of skin wrinkle- The wrinkle reduced property was evaluated by measuring the area of wrinkle alternatively before applying the cream. The length of the ageing was measured using a scale and transparent paper by placing the paper on ageing and tracing it out on alternate day. Significant decrease in area of the ageing (around 0.33 mm in every 5th day) in the test group of animals as compare with the marketed and negative control groups animal, it indicates that formulated cream is effective for ageing treatment, the area of ageing in test group decreases enough to justify the effectiveness of the formulated cream of pomegranate seed oil.

Table- 6 Area of skin wrinkle

Animal group	0th day	5th day	10th day	15th day	20th day	25th day	30th day	35th day
Negative control	1.5cm	1.5 cm	1.4 cm	1.4 cm	1.4 cm	1.3 cm	1.2 cm	1.1 cm
Standard	1.5cm	1.1 cm	0.7 cm	0.3 cm	0.2 cm	-	-	-
Test	1.5cm	1.3 cm	0.8 cm	0.5 cm	0.2 cm	0.2 cm	-	-

Table7: skin irritancy scoring table

Score	Reaction
0	No
1	Slightly
2	Moderate
3	Severe

Fig 6: Animal were used to evaluate skin irritancy(F5)

Remove hair	Apply cream	No skin irritation
		

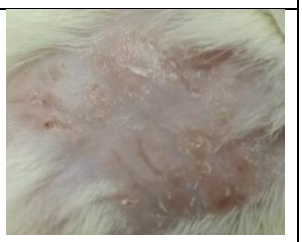
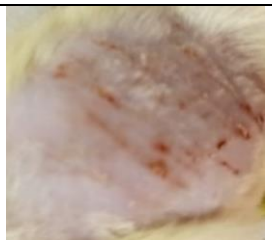
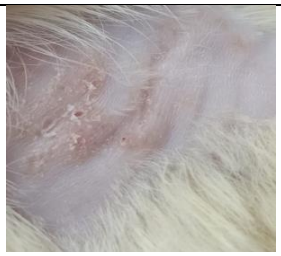
3. Allergic response- The symptoms allergic response includes rash, redness, swelling, cracked skin, and itching. When conducting the study, these elements were considered. Four animals in all were used in the investigation. Dorsal skin hair was removed using hair removal cream the formulated cream was applied on 1.5cm skin area for 10 days. On the basis of a scoring table, it was appraised. They are indicated by table number 8. The allergic response study's final finding were noted and reported in table number 8.

Table 8: Allergic response

Score	Allergic symptoms	Reaction
0	Rash	No
1	Redness	Slightly
2	Swelling	No
3	Cracked skin	No
4	Itching	Moderate

4. Photographic comparison

After creating an ageing according to the guidelines of the CPCSEA, photographs of each group of animal's ageing part were taken for the visual comparison, and the same is shown in figure7: Following formulation applied to the ageing each group of animals.

Day	First Group	Second Group	Third Group	Forth Group
0				
5				
10				
15				
20				
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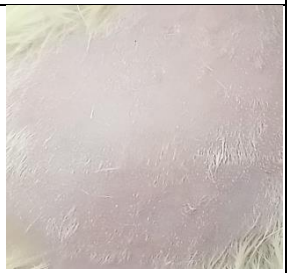
30				

Fig:7-Top-view images of ageing on days 0, 5, 10, 15, 20, 25, and 30 Where - Group 1 is the control group, Group 2 is the negative control group(induced ageing by uv light after 10 days we can follow the group3), Group 3 is the marketing group, and Group 4 is the test group

IN VIVO STUDY

1. Area of skin wrinkles- The wrinkle reduced property was evaluated by measuring the area of wrinkle alternatively before applying the cream. The length of the ageing was measured using a scale and transparent paper by placing the paper on ageing and tracing it out on alternate day. Significant decrease in area of the ageing (around 0.33 mm in every 5^{th} day) in the test group of animals as compare with the marketed and negative control groups animal, it indicates that formulated cream is effective for ageing treatment, the area of ageing in test group decreases enough to justify the effectiveness of the formulated cream of pomegranate seed oil.

2. Study on skin irritation - The symptoms of rashes on skin studied includes Redness, itching, oedema, discomfort, and sensitivity. When conducting the study, these elements were considered. Four animals in all were used in the investigation. Dorsal skin hair was removed using hair removal cream and the formulated cream was applied for 10 days. On the basis of a scoring table, it was appraised. They are indicated by table number13. The skin sensitivity study's final finding were noted and reported in table number 13.

3. Allergic response- The symptoms allergic response includes rash, redness, swelling, cracked skin, and itching. When conducting the study, these elements were considered. Four animals in all were used in the investigation. Dorsal skin hair was removed using hair removal cream the formulated cream was applied on 1.5cm skin area for 10 days. On the basis of a scoring table, it was appraised. They are indicated by table number 15. The allergic response study's final finding were noted and reported in table number 15.

4. Photographic observation and comparison- After creating a ageing according to the guidelines of the CPCSEA, the images was captured of each animal groups for the visual comparison, and those image was attached in figure number.. It was observed that anti-ageing formulated cream of pomegranate seed oil showed nearly similar ageing healing as compared to the marketed anti-ageing cream. The ageing of test group animals marketed control group animals was healed completely on the 30^{th} day, and the ageing of negative control group animals was not healed till the last day of study.
A comparative study of marketed and formulated anti-ageing cream, after performing all the evaluation parameters, the comparison are given in table number9.

Table 9: Comparison between marketed and formulated anti-ageing cream

Sl. No.	Marketed cream	Formulated cream
1	It is polyherbal formulation	It is single herb used
2	It is causes mild irritation after applying on skin	No irritation on skin
3	Need to apply 2-4 times a day	Two times a day
4	This is high cost cream	Low cost cream
5	Need at least 2 month for new ageing and takes 8 months to complete healing of old ageing	It is expected to take 2 month for new ageing

Conclusion

Our work's objective was to create, improve, and assess an anti-aging cream that included pomegranate seed oil, it includes ageing that are formed on our skin during the natural healing process of body cells. Ageing are developed in our body, during UV radiation, sunburn and environment pollution in our skin the immune system and natural healing process of our body activates immediately and send messages to form fresh collagen fibres and attack the infection. This results into the developments of ageing.
Vanishing cream of pomegranate seed oil was ready successfully using several substances, including potassium hydroxide, cetyl alcohol, and stearic acid, glycerine, perfume water and anti-microbial agents, The reason vanishing cream was chosen is that it left a thin coating on the skin that lets the cream stay on the skin longer, which ultimately results within significant reduction of ageing. In the study oil-in-water cream formulation was selected because They consist of tiny oil droplets scattered throughout a continuous water phase and the proposed cream

formulation would be more comfortable and cosmetically for skin ageing as It is easier to remove with water and less oily.

The prepared cream formulation was analysed for different in-vitro and in-vivo parameters such as Organoleptic parameters, types of emulsion test, viscosity and Spreadibility for in vitro and area of skin wrinkles, skin irritation, allergic response, skin texture and photographic comparison for in-vivo evaluation and stability study was also performed. The range of 3260 to 6499 Cp was discovered to be the viscosity of the formulated cream. The Spreadibility was found in the range of 21to 30 gm.cm/sec, pH was recorded at 6.5 constantly for 3 months which shows the stability of the cream.

In the animal study, healthy albino rats (150-300g) were chosen to perform every parameters of in-vivo research. Study was performed at Truba institute of Pharmacy, Bhopal (M.P.) in division of pharmacology lab with appropriate institutional authorization Animal Ethical Committee(IAEC approval no. PH/IAEC/2K23). An in-vivo study's findings showed that the ageing regulated by formulated pomegranate seed oil cream, recovery 100% with each better skin appearance and the ageing of test group was healed completely on the 30^{th} Dy. It was observed that formulated cream of pomegranate seed oil showed nearly similar ageing healing as compared to the marketed anti-ageing cream.

After performing different in-vitro, in-vivo and stability study parameters, it was observed that pomegranate seed oil was effective as a cream formulation to treat the skin ageing, skin wrinkles, fine line, dark spots and it also significant effectiveness against sunburn. Results of the study suggest that the formulated anti-ageing cream of pomegranate seed oil and its oil was safe, stable and cost effective for topical cosmetic formulation.

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