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Formulation and evaluation of Antiwrinkle activity of Cream and Nano emulsion of Moringa oleifera seed oil

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Abstract: *Aging is a natural phenomenon that leads to various changes in the physiology of the skin. The changes occurring in the physiology of the skin makes the candidate to appear old. Application of antiaging creams is the best choice even though various treatment methods are available because it nourishes skin and prevents or repairs fine lines and wrinkles thus giving young looking appearance. Apart from that, nature has an excellent anti-aging remedies that acts externally whereas internally to delay aging signs and some will act to repair and prevent aging signs. In the present study, Moringa oleifera was studied for its antiaging benefits as the seed oil is rich in antioxidants that might prevent the oxidative damage of the skin. By using the Moringa seed oil in various ratios, cream and nano emulsion were prepared and they are characterised for its physical properties. The best formula was optimised which has been evaluated for antiaging activity using animal models by topical application of the formulations for two times a day upto 30days and the results were compared with the standard. The results showed that the nano emulsion formulation was found more efficacious than the cream formulation. This shows that the moringa oil has a good antiaging activity and donot showed any irritant effects on skin.*

Key Words: *Wrinkles, Premature skin aging, Moringa oleifera, Oxidative damage, Skin topography analysis*.

I. Introduction

Aging can be noticed by seven key signs like fine lines and wrinkles, changes in skin tone and texture, skin surface dullness, visible pores, Blotchiness, age spots and Dryness. Among all these signs, appearance of fine lines and wrinkles on skin is the common and most prominent sign of aging. So skin creams used to prevent aging signs are also called as Anti-wrinkle creams. Skin consists of three layers Epidermis, Dermis and Subcutaneous layer. Dermis of the skin consists of elastin fibres which will maintains the skin structure by stretching and folding back when the muscle undergo various stress conditions like facial expressions etc. Collagen, also present in Dermis is responsible for preventing wrinkle formation. Upon aging, the skin will lose elastin and also Collagen undergoes breakdown[1]. This makes the skin thinner and moisture cannot enter the skin layers making the skin drier. The subcutaneous fat which gives the skin the plumpy appearance also begins to disappear. All these conditions will leads to progression of wrinkle formation which is due to the structural changes in lower dermal layers of the skin[2] which is shown in the **table 1 and figure 1.**

Aging is of two types: Chronological aging or Intrinsic aging and Photo aging[3]. Photo-aging also called as premature skin aging[4] is caused by continuous exposure of the skin to solar UV irradiation. It results in several skin symptoms, such as leathery texture, mottled pigmentation, and wrinkles**.** Apart from Intrinsic and photoaging, so called stochastic aging[5] connotes cell damage by metabolic processes, free radicals and cosmic irradiation. Hence, reducing oxidative stress has been the major focus of anti-aging research, and the antioxidant supplements are recommended based on this research. Moreover, these antioxidants are active both topically and internally, which means they can be applied in form of creams, gels, or taken as capsules or tablets. Anti-oxidants are helpful to achieve efficient free radical scavenging, since they relieve one another of free radical burden. Vitamin E, Vitamin C, lipoic acid, coenzyme Q10, nicotinic acid, and glutathione neutralize free radicals by different methods, and they complement one another's efforts.

Based on this data[5], Moringa oleifera was choosed for the present study inorder to evaluate the antiwrinkle efficacy of the seed oil which is rich in antioxidants[7]. Using the seed oil of M. Oleifera, cream and nano-emulsion were prepared and studied for antiwrinkle efficacy. As the prepared formulation is a herbal formulation it may reduce the side effects of the chemical based marketed formulations.

II. Materials and Methods:

2.1. Plant Material: The seeds of *Moringa oleifera* Fam (Moringaceae) were collected from local region of Vijayawada, Andhra Pradesh, India, in june 2013. The plant material was identified and authenticated by Dr. P. G. Diwakar Botanical survey of India, Hyderabad (Ref no. BSI/WC/Tech/2013 /370).

Moringa oleifera seeds (**Figure 2**) were collected from surroundings of Vijayawada. Pods and shells were removed manually and kernels were grounded in a domestic blender (Preethi) and sieved through 600 µm stainless steel sieve. Solvent selected for extraction of oil was 95% ethanol (Et).

2.2. Oil Extraction procedure:

Manual (man): *Moringa oleifera* seeds were defatted by using 95% ethanol in 5 % (w/v) suspension, mixing with a magnetic stirrer for 60 minutes. Supernatant was separated by centrifugation (3000 rpm, 45 min) and the settled powder was dried at room temperature for 24 hours.

Continues (Sx)*:* About 50 g of *M. oleifera* crushed seeds were fed to a lab-scale Soxhlet extractor fitted with a 1-L round-bottom flask and a condenser. The extraction was executed for 6 hours with 350 mL of solvent. The extracted oil yield was expressed as percentage, which is defined as weight of oil extracted over weight of the sample taken.

2.3. Cream formulation: Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (Cetyl alcohol, liquid paraffin) were dissolved in the oil phase (Part A) and heated to 75° C. The preservatives and other water soluble components (Methyl paraben, Glycerol, Propylene glycol, ethanol extract of *Moringa oleifera* seeds were dissolved in the aqueous phase (Part B) and heated to 75° C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifier took place. The formula for the cream was given in **table 2.** The formulated cream was subjected to the following evaluation parameters such as pH determination, viscosity, Dye test, Homogenity, appearence, after feel, Type of smear, removal, acid value, saponification value, irritancy test and Accelerated stability analysis.

2.4. Preparation of *Moringa oleifera* seed oil nano-emulsion (MONE)

An emulsion was prepared following the formula in the ingredient percentages (**Table 3).** The following materials were used to produce the emulsion; Water, *Moringa oleifera* seed oil, and Sodium caseinate. Sodium caseinate was used since it is a natural emulsifier (flexible protein) which has been used to produce nano-emulsions. Addition rate (% w/w) of the ingredients was modified to emulsify the highest percent of MONE and produce the lowest droplet size (nm) without affecting the emulsion stability. Sodium caseinate was dissolved in water and stirred using a magnetic stirrer at 700 rpm for 1 hr. at room temperature. *Moringa oleifera* seed oil was added gradually into the sodium caseinate solution and mixed by stirring for 5 min. An ultrasonic processor set at 80% amplitude was used to produce a stable micro-emulsion to process each batch (100 mL/batch) of solution in a 250 mL glass beaker for about 10 min. The emulsion was constantly cooled to maintain a temperature of approximately 20 °C by means of immersion in an ice bath during the sonication process. The emulsion was then processed by a mechanical stirrer device to produce a nano-emulsion. Emulsion batches (100 mL/batch) in a 200 mL beaker was sheared at 16,000 rpm for 10 min to reduce droplet size to nano-scale. The beaker was set in an ice bath to maintain a temperature of approximately 20 °C during mechanical stirring. An emulsion produced only by ultrasonication was prepared as a control. The nano-emulsion and the control emulsion were kept refrigerated at 5 °C[7]. Composition *Moringa oleifera* Nano-emulsion Formulations was given in the **Table 3.** It was characterized for the following parameters such as particle size, Particle surface charge (zeta potential), transmission electron microscopic studies (TEM).

2.5. Evaluation: By performing the above mentioned evaluation tests, the best formula was optimised, and it was subjected to further evaluation of antiwrinkle activity by using animal models. Various tests that can be performed to evaluate the antiwinkle efficacy are: Wrinkle score measurement, Histological evaluation , Invitro trial - Hydrating and Anti-Wrinkle efficacy, Staining, Anti-wrinkle test on healthy mice using silicon imprints, Instrumental assessment using tewameter MPA 5, Skin moisture and TEWL, Surface Evaluation of Living Skin (SELS), volume and energy which can be studied using Visioscan® VC 98/ software SELS 2000.

In the present study the following method was used for evaluating the antiwrinkle efficacy of the prepared formulation:

Study of Anti-wrinkle efficacy using animal models by analysis of Photographs and by Staining method: (Test was performed at **Albino research and training institute, Hyderabad, Andhra pradesh, India)** Four-week-old female hairless mice (SKH-1) weighing 17–24 g were obtained from Indian Institute of Toxicology Research Lucknow. Mice had free access to food and water and were acclimated to the air-conditioned room (23 ± 2°C and 50 ± 10% humidity with a 12 h light/12 h dark cycle) for 1 week before the *in vivo* anti-wrinkle study.

Firstly, the animals under study are subjected to UVB irradiation at suberythemal doses for upto 2weeks at 345nm which will produce marked wrinkling[8,9,10]. Skin topography analysis was performed on the skin surface of mice before and after treatment for measuring the effectiveness of *M. Olifera*. The same skin areas were examined before the process (Day 0) and after the process (Day1, Day7, Day15 and Day 30), by taking photographs and also by means of observation under magnifying glass (Leica M LZIII, augment 1OX) with outer white light.

The mouse was divided into two groups, those with male mouse and those with female mouse, 50% of the mouse in the study are male and the other 50% female. The test was performed under the environmental conditions between 23-25ºC and relative humidity of 60%.

Two types of trials were carried out:

1. It was studied whether the two formulations could affect the aqueous content of the skin and
2. The evaluation of the wrinkle, in the short-term and long-term study.

Short-term trial: the formulas were applied for every 12 hours and measurements were taken at the end of 24 hours after application.

Long-term trial: To do this, the subjects were treated with the cream for one month, twice a day. The evaluation of the aqueous content of the skin was determined after 24 hours , 7, 15, 21 and 30 days.

In the present study only the wrinkle evaluation was done in long-term study. The appearance of the skin depends on its general condition, and in particular its level of moisturisation, as this regulates the elasticity, flexibility and smoothness of the skin. The integrity of the extracellular matrix, especially the collagen fibres, is essential to ensure that the epidermis is firmly anchored to the dermis. It has been suggested that the deterioration of the collagen fibres, at the dermis-epidermis union, weakens it, which finally leads to the appearance of wrinkles. In expression wrinkles, the contraction of the muscle leads to contraction of the fibroblasts and with this the contraction of the collagen fibres occurs and even deterioration of the extracellular matrix also occurs in the affected area. Consequently, the hydrating properties of all the components of the matrix will be affected.

Antiwrinkle creams should be capable of relaxing the fibroblasts, which will decontract the collagen and elastin matrix, which could be decisive in preventing deterioration of the functions of collagen, such as water retention. Thus, it could be deduced that by reducing the wrinkle, greater moisturisation is achieved.

Photographs of a wrinkle of mouse with male and female, respectively, are taken in the beginning and upto 30 days of treatment. In the photographs, it is possible to observe that the formulations under study improved the appearance of the wrinkles.

After 30 days treatment with the topical preparations, the photographs were observed (**Figure-8 and figure-11)**. There was no skin changes in **Group-1** animals. In **Group-2** (negative control, saline with UV irradiation) thick and deep wrinkles were observed. In contrast, **Group-3** (positive control, RESIST Intensive Wrinkle-Repair Retinol Serum with UV irradiation) showed better anti-wrinkle scores. The **Group-4** (MONE 3 with UV irradiation) showed significantly better anti-wrinkle scores compared with standard anti wrinkle cream. The **Group-5** (Formulation 3 *Moringa oleifera* Oil based cream with UV irradiation) showed smooth and improved skin surfaces. Results after the 30day study of all the groups was shown in the

III. Results and Discussions:

The result data was tabulated in **Table No: 4- 12** and shown in **Figure No: 3-11.**

Table 1: Skin structure and their observed effects of aging[1]

Skin structure	Observed effects of aging
	Lower lipid content was observed
Epidermis	1. Dermal-epidermal junction flattens 2. Number of enzymatically active melanocytes decreases by 8% to 20% per decade 3. Number of langerhans cells decreases 4. Capacity for re-epithelization diminishes 5. Number of pores increases
Dermis	1. Thickness reduced (atrophy) 2. Vascularity and cellularity decrease 3. Collagen synthesis decreases 4. Pacinian and meissner's corpuscles degenerate 5. Structure of sweat glands becomes distorted, number of functional sweat glands of decreases

	6. Elastic fibers degrade 7. Number of blood vessels decreases and number of nerve endings reduced
Hypodermis	Distribution of subcutaneous fat changes and overall volume of fat decreases.
Appendages	1. Number of sweat glands decreases 2. Nail plates become abnormal 3. Sebum production reduced 4. Hair loses normal pigments and thins up

Table 2: Composition *Moringa oleifera* oil based cream

S.No	Ingredients	Formula % w/w				
		F1	F2	F3	F4	F5
1.	Extracted seed oil of *Moringa oleifera*	2	2	2	2	2
2.	Stearic acid	10	8	6	4	2
3.	Cetyl alcohol	6	6	6	6	6
4.	Liquid paraffin	6.6	6.6	6.6	6.6	6.6
5.	Glycerol	3	3	3	3	3
6.	Methyl paraban	0.02	0.02	0.02	0.02	0.02
7.	Propylene glycol	30	30	30	30	30
8.	Water, qs, 100	Qs	qs	qs	qs	qs

Table 3: Composition *Moringa oleifera* Nano-emulsion Formulations

S.No	Ingredients	Amount (%)*				
		MONE 1	MONE 2	MONE 3	MONE 4	MONE 5
1.	***Moringa oleifera* oil**	5	10	15	20	25
2.	Sodium caseinate	10	10	10	10	10
3.	Water	85	80	75	70	65
4.	Total	100	100	100	100	100

*Mass basis

Table 4: Oil yield extraction for both methods and solvents

S.No	Method	% of Seed Oil yield extraction (w/w)
1.	Manual extraction	17.81
2.	Soxhlet extraction	32.11

Table 5: a:Physical parameter of F1 cream on room and accelerated temperature

Formulation	Days	Temperature	pH	Homogeneity	Appearance	Spreadibility	After feel	Type of smear	Removal
F1	0	RT	5.7	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.6	Good	No change in colour	Good	Emollient	Non greasy	Easy
	5	RT	5.7	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.6	Good	No change in colour	Good	Emollient	Non greasy	Easy
	10	RT	5.8	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.7	Satisfactory	No change in colour	Good	Emollient	Non greasy	Easy
	15	RT	5.9	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.7	Satisfactor	No change	Good	Emollient	Non	Easy

				y	in colour			greasy	
	20	RT	5.9	Good	No change in colour	Low	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.8	Satisfactor y	No change in colour	Low	Emollient	Non greasy	Easy

Table 5: b:Physical parameter of F2 cream on room and accelerated temperature

Formulatio n	Days	Temperatur e	pH	Homogenity	Appearance	Spreadibilit y	After feel	Type of smear	Removal
F2	0	RT	5.8	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.7	Good	No change in colour	Good	Emollient	Non greasy	Easy
	5	RT	5.8	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.7	Good	No change in colour	Good	Emollient	Non greasy	Easy
	10	RT	5.9	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.7	Good	No change in colour	Good	Emollient	Non greasy	Easy
	15	RT	5.9	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.8	Satisfactory	No change in colour	Good	Emollient	Non greasy	Easy
	20	RT	6.0	Good	No change in colour	Low	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.8	Satisfactory	No change in colour	Low	Emollient	Non greasy	Easy

Table 5:c: Physical parameter of F3 cream on room and accelerated temperature

Formulation	Days	Temperature	pH	Homogenity	Appearance	Spreadibility	After feel	Type of smear	Removal
F3	0	RT	6.0	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.8	Good	No change in colour	Good	Emollient	Non greasy	Easy
	5	RT	6.1	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.7	Good	No change in colour	Good	Emollient	Non greasy	Easy
	10	RT	6.2	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.9	Good	No change in colour	Good	Emollient	Non greasy	Easy
	15	RT	6.2	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	5.9	Good	No change in colour	Good	Emollient	Non greasy	Easy
	20	RT	6.3	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.0	Good	No change in colour	Good	Emollient	Non greasy	Easy

Table 5:d: Physical parameter of F4 cream on room and accelerated temperature

Formulation	Days	Temperature	pH	Homogenity	Appearance	Spreadibility	After feel	Type of smear	Removal
F4	0	RT	6.6	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.4	Good	No change in colour	Good	Emollient	Non greasy	Easy
	5	RT	6.7	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.6	Good	No change in colour	Good	Emollient	Non greasy	Easy
	10	RT	6.8	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.7	Satisfactory	No change in colour	Good	Emollient	Non greasy	Easy
	15	RT	6.8	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.8	Satisfactory	No change in colour	Poor	Emollient	Non greasy	Easy
	20	RT	6.9	Good	No change in colour	Poor	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.8	Satisfactory	No change in colour	Poor	Emollient	Non greasy	Easy

Table 5:e: Physical parameter of F5 cream on room and accelerated temperature

Formulation	Days	Temperature	pH	Homogenity	Appearance	Spreadibility	After feel	Type of smear	Removal
F5	0	RT	6.7	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.5	Good	No change in colour	Good	Emollient	Non greasy	Easy
	5	RT	6.8	Good	No change in colour	Good	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.6	Satisfactory	No change in colour	Good	Emollient	Non greasy	Easy
	10	RT	6.9	Good	No change in colour	Low	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.7	Satisfactory	No change in colour	Low	Emollient	Non greasy	Easy
	15	RT	7.1	Good	No change in colour	Low	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.8	Satisfactory	No change in colour	Low	Emollient	Non greasy	Easy
	20	RT	7.1	Good	No change in colour	Poor	Emollient	Non greasy	Easy
		40 ºC ± 1 ºC	6.8	Satisfactory	No change in colour	Good	Emollient	Non greasy	Easy

Table 6: Type of Adverse effect of formulations

S.No	Formulation	Irritant	Erythema	Edema

1.	**F1**	NIL	NIL	NIL
2.	**F2**	NIL	NIL	NIL
3.	**F3**	NIL	NIL	NIL
4.	**F4**	NIL	NIL	NIL
5.	**F5**	NIL	NIL	NIL

Table 7: Test for acid value and saponification value

S.No	Formulation	Acid value	Saponification value
1.	**F1**	6.7	29.2
2.	**F2**	6.4	27.5
3.	**F3**	5.7	25.6
4.	**F4**	5.5	22.1
5.	**F5**	6.4	21.3

Table 8: Rheological study

Formulations	Days	Viscosity (cps)	
		Room temperature	45°C
1.	**0**	27500	27000
2.	**5**	28500	26000
3.	**10**	30000	24000
4.	**15**	31000	22500
5.	**20**	32000	21000

Table 9: *Moringa oleifera* nanoemulsion Mean particle diameters (nm) and (PDI)

S.No	Formulation code	Mean particle diameters (nm)	Polydispersity Index (PDI)
1.	MONE 1	67 ± 5	0.36 ± 0.03
2.	MONE 2	57 ± 4	0.28 ± 0.01
3.	MONE 3	51 ± 1	0.22 ± 0.04
4.	MONE 4	53 ± 5	0.37 ± 0.06
5.	MONE 5	62 ± 2	0.24 ± 0.05

Table 10: *Moringa oleifera* nanoemulsions zeta potential (mV)

S.No	Formulation code	zeta potential (mV)
1.	MONE 1	29 ± 2.1
2.	MONE 2	31 ± 4.2
3.	MONE 3	43 ± 1.5
4.	MONE 4	39 ± 2.5
5.	MONE 5	35 ± 3.4

Table 11-a: Analysis of the visual evaluation of the reduction in number of wrinkles in 3 cm square area after applying *M.olifera* oil nanoemulsion formulation.

Time (days)	0		1		7		15		30	
Mouse	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**
					Number of wrinkles count					
Group 1 (Control)	13	13	10	10	10	11	10	11	10	11
Group 2	15	13	13	12	12	11	11	10	08	08
Group 3	13	12	12	11	11	10	10	09	09	08
Group 4	12	13	10	12	10	11	09	10	07	08
Group 5	16	15	15	15	15	14	13	12	11	10
Group 6 (Standard)	15	17	12	13	11	12	08	10	05	05

Table 11-b: Analysis of the visual evaluation of the reduction in number of wrinkles in 1 cm square area after applying *M.olifera* oil cream formulation.

Time (days)	0		1		7		15		30	
Mouse	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**
					Number of wrinkles count					
Group 1 (Control)	10	11	10	11	09	09	08	08	07	07
Group 2	13	12	13	12	12	11	11	10	09	09
Group 3	12	12	12	11	11	10	10	09	09	08
Group 4	10	13	10	12	10	11	09	10	08	08
Group 5	15	16	15	15	15	14	13	13	12	11
Group 6 (Standard)	14	17	12	13	11	12	08	10	05	05

Table 12-a: Shows analysis of the visual evaluation of the reduction in size of wrinkles (mm) in 3 cm square area after applying *M.olifera* oil nanoemulsion formulation.

Time (days)	0		1		7		15		30	
Mouse	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**
					Number of wrinkles count					
Group 1 (Control)	9.0± 0.03	9.0± 0.05	9.0± 0.17	9.0± 0.15	9.0± 0.33	9.0± 0.15	9.0± 0.13	9.10± 0.09	9.0± 0.12	9.0± 0.35
Group 2	9.0± 0.07	9.0± 0.11	8.0± 0.09	8 0± 0.10	7.0± 0.12	7.0± 0.14	6.0± 0.16	6.10± 0.15	5.0± 0.17	6.0± 0.12
Group 3	13.0± 0.04	12.0± 0.10	13.0± 0.07	12 0± 0.13	11.0± 0.33	10.0± 0.09	9.0± 0.13	9.10± 0.23	7.0± 0.15	5.0± 0.15
Group 4	15.0± 0.12	13.0± 0.15	14.0± 0.06	13 0± 0.15	13.0± 0.16	12.0± 0.15	12.0± 0.19	10.10± 0.09	8.0± 0.12	8.0± 0.11
Group 5	14.0± 0.15	12.0± 0.22	14.0± 0.10	12 0± 0.11	13.0± 0.54	11.0± 0.08	12.0± 0.09	10.10± 0.09	11.0± 0.19	10.0± 0.35
Group 6 (Standard)	10.0± 0.11	10.0± 0.21	10.0± 0.10	10 0± 0.19	9.0± 0.30	7.0± 0.18	6.0± 0.05	6.10± 0.25	5.0± 0.12	5.0± 0.32

Table 12-b: Shows analysis of the visual evaluation of the reduction in size of wrinkles (mm) in 1 cm square area after applying *M.olifera* oil cream formulation.

Time (days)	0		1		7		15		30	
Mouse	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**	**Male mouse**	**Female mouse**
					Number of wrinkles count					
Group 1 (Control)	8.0± 0.13	12.0± 0.07	8.0± 0.11	12.0± 0.10	8.0± 0.12	12.0± 0.15	8.0± 0.14	12.10± 0.02	9.0± 0.11	14.0± 0.30
Group 2	10.0± 0.11	11.0± 0.08	8.0± 0.09	8.0± 0.15	7.0± 0.33	7.0± 0.10	6.0± 0.16	6.10± 0.09	5.0± 0.12	6.0± 0.12
Group 3	12.0± 0.21	13.0± 0.18	12.0± 0.07	12.0± 0.13	11.0± 0.12	10.0± 0.15	9.0± 0.13	9.10± 0.05	7.0± 0.12	7.0± 0.35
Group 4	13.0± 0.33	12.0± 0.16	14.0± 0.06	13.0± 0.11	13.0± 0.33	12.0± 0.18	12.0± 0.13	10.10± 0.09	10.0± 0.15	10.0± 0.05
Group 5	13.0± 0.11	12.0± 0.11	13.0± 0.07	12.0± 0.15	13.0± 0.54	11.0± 0.15	12.0± 0.19	10.10± 0.10	11.0± 0.12	10.0± 0.35
Group 6 (Standard)	11.0± 0.09	11.0± 0.43	11.0± 0.15	10.0± 0.10	9.0± 0.33	10.0± 0.16	6.0± 0.10	6.0± 0.09	5.0± 0.4	5.0± 0.09

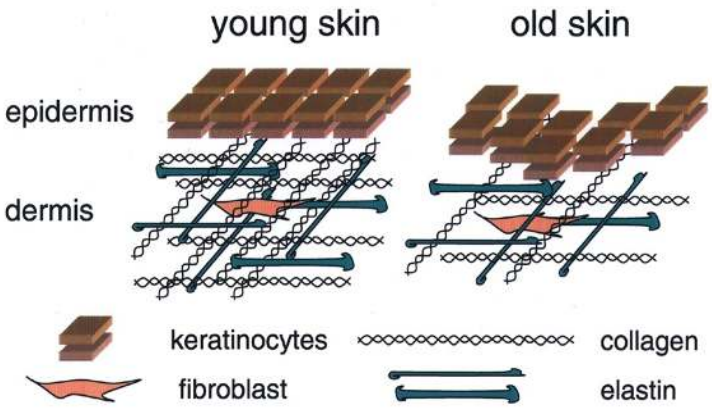


Figure 1: Schematic drawing of young and old skin.

Figure 2: ***Moringa oleifera*** **seeds as collected from the fruit**

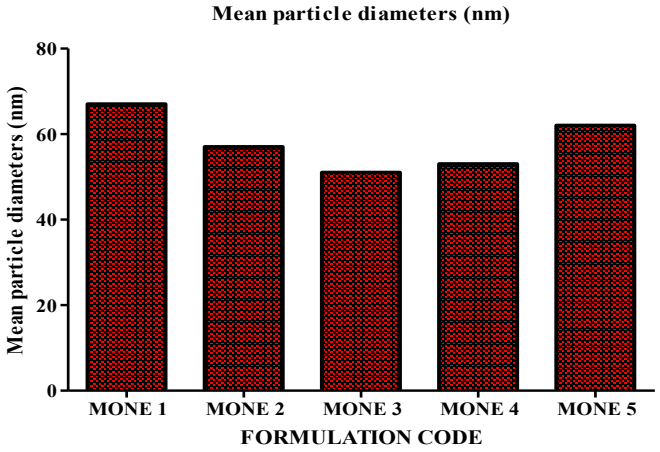


Figure 3: ***Moringa oleifera*** **nanoemulsions Mean particle diameters (nm)**

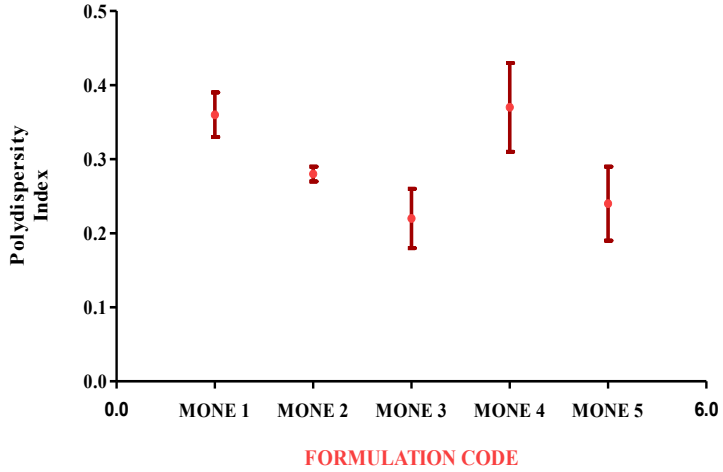


Figure 4: *Moringa oleifera* nanoemulsions Polydispersity Index (PDI)

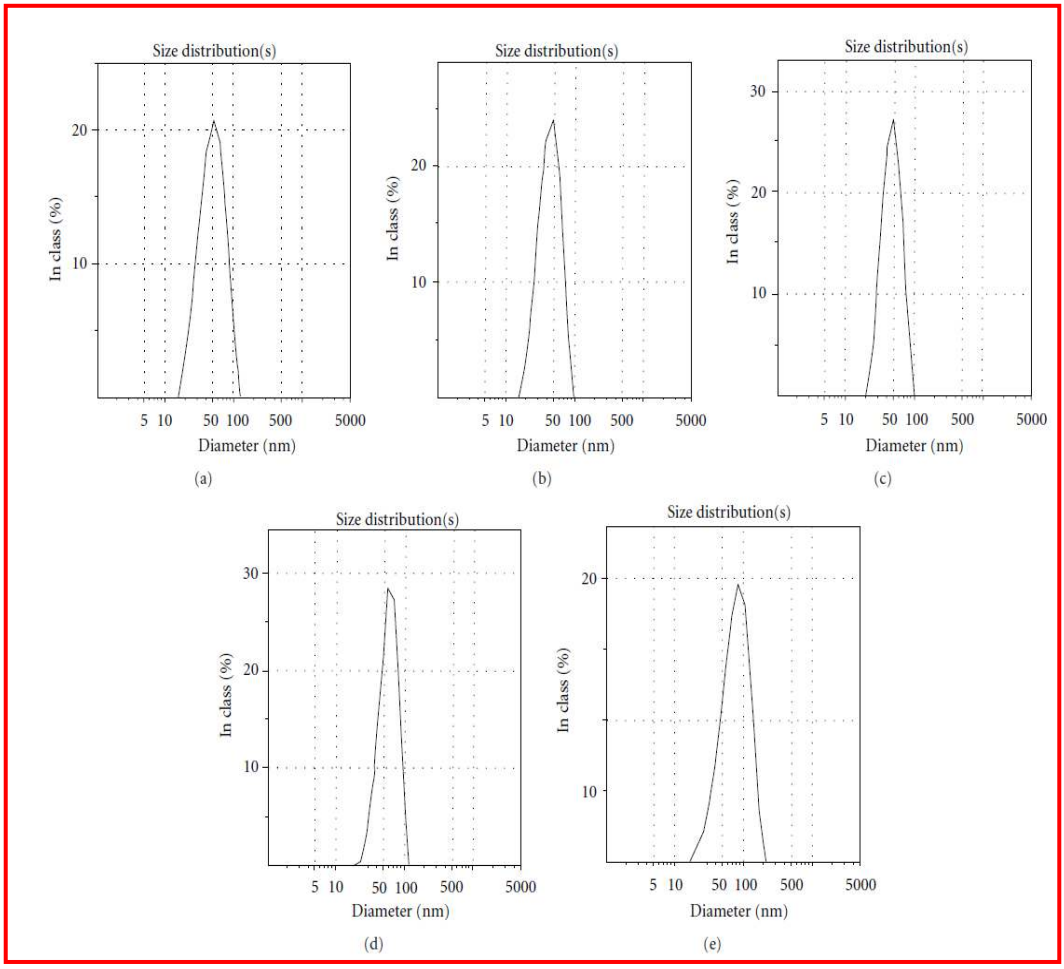


Figure 5: *Moringa oleifera* nanoemulsions mean particle diameters (nm), a: MONE 1 b: MONE 2, c: MONE 3, d: MONE 4, e: MONE 5.

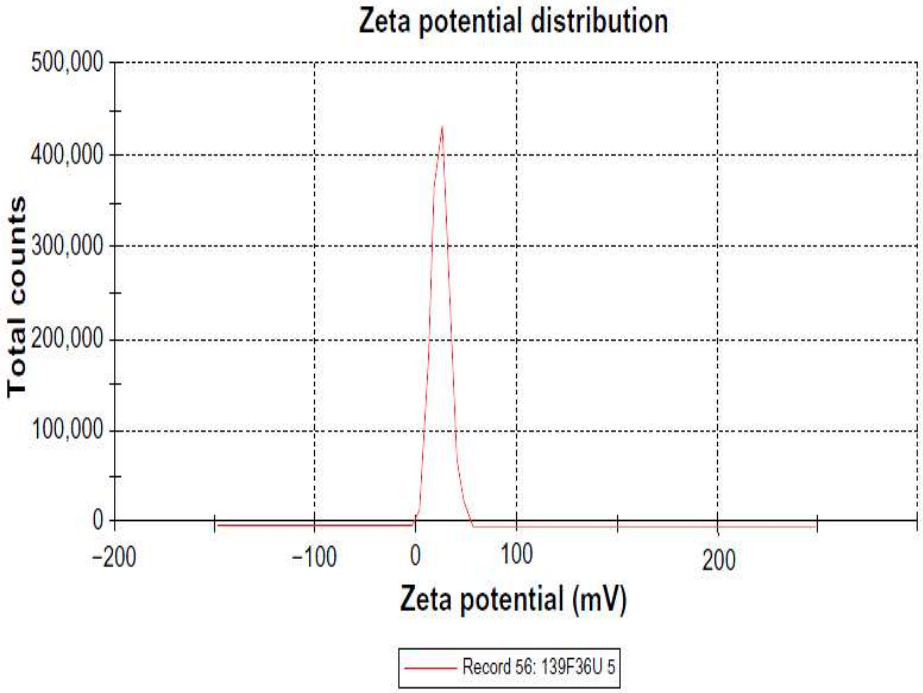


Figure 6: MONE 3 zeta potential (mV)

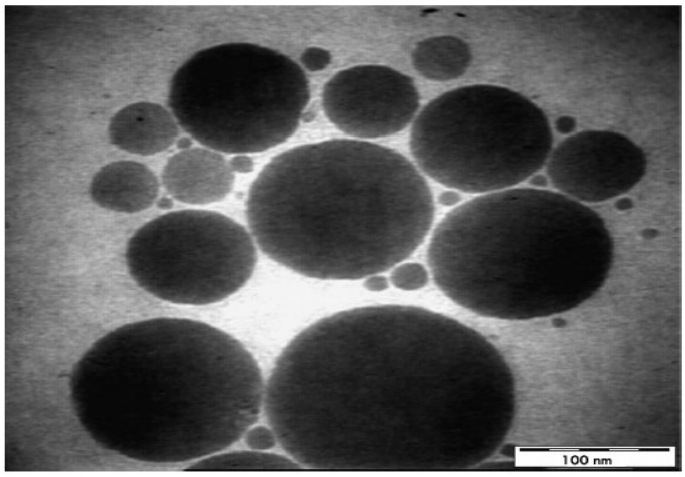
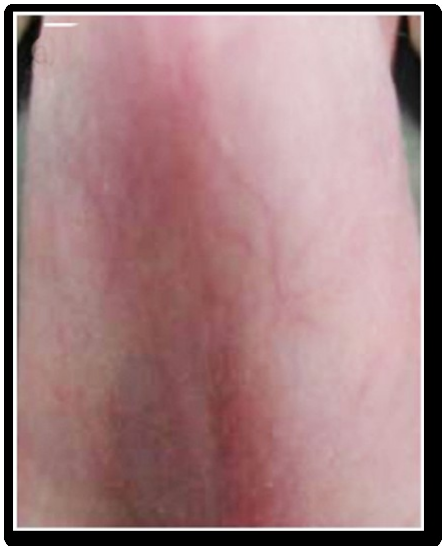
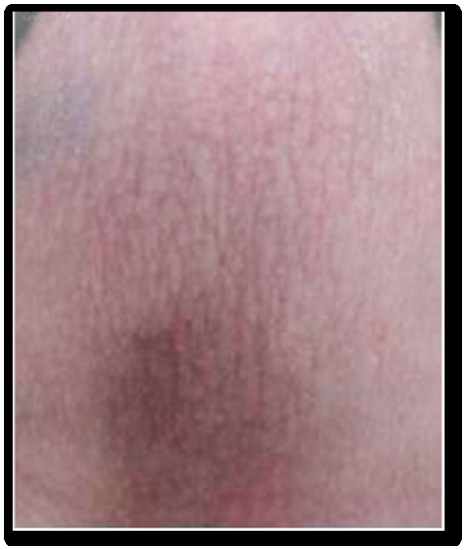


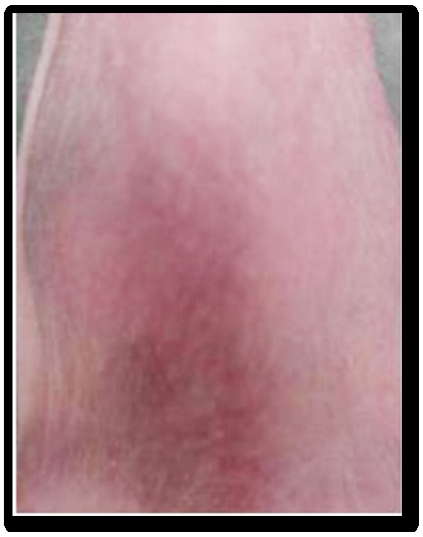
Figure 7: TEM photographs of the MONE 3



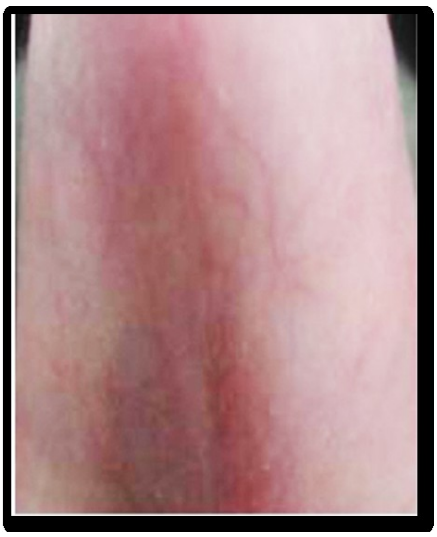
Group 1: normal skin



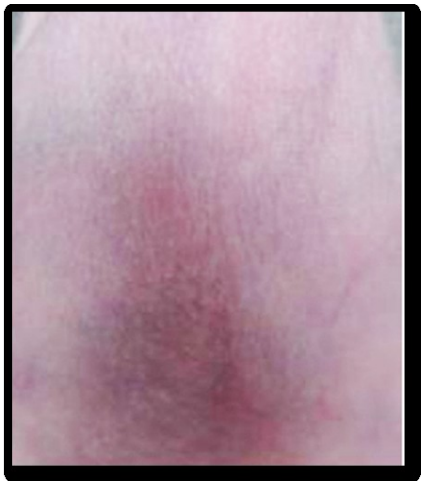
Group 2: Thick and deep wrinkles were observed



Group 3: showed very smooth skin surface



Group 4: showed very smooth skin surface



Group 5: showed very smooth skin surface

Figure 8: Anti - wrinkle score of experimental animals

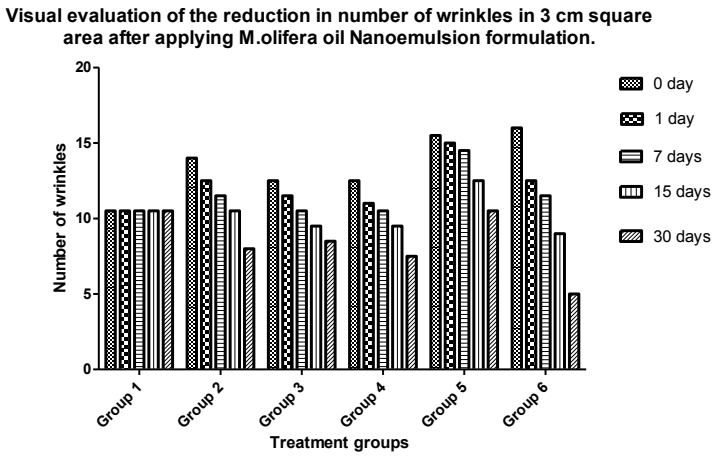


Figure 9-a: Shows analysis of the visual evaluation of the reduction in number of wrinkles in 3 cm square area after applying *M.olifera* oil nanoemulsion formulation.

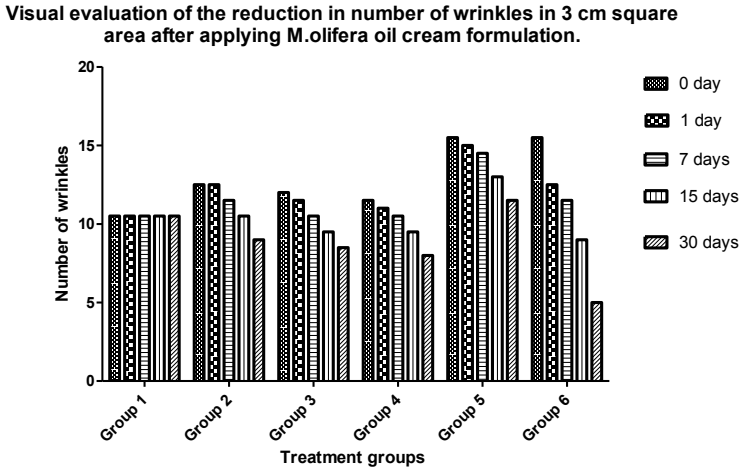


Figure 9-b: Shows analysis of the visual evaluation of the reduction in number of wrinkles in 3 cm square area after applying *M.olifera* oil cream formulation.

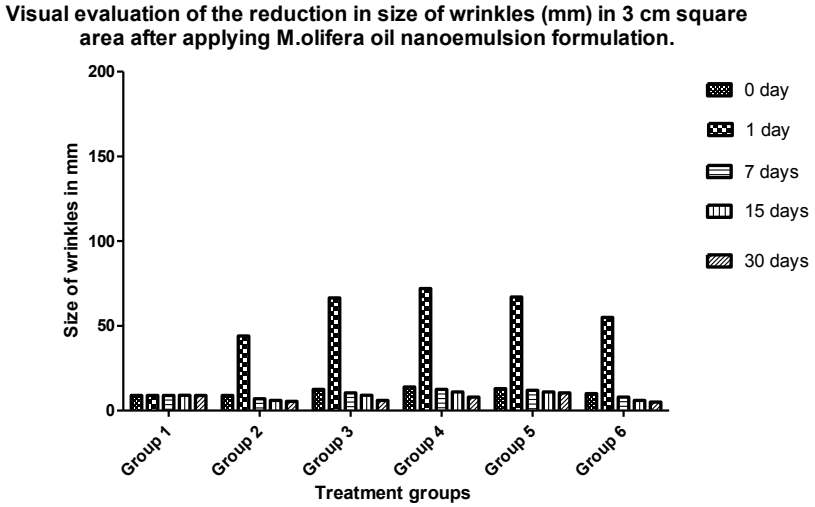


Figure 10-a: Shows analysis of the visual evaluation of the reduction in size of wrinkles (mm) in 3 cm square area after applying *M.olifera* oil nanoemulsion formulation.

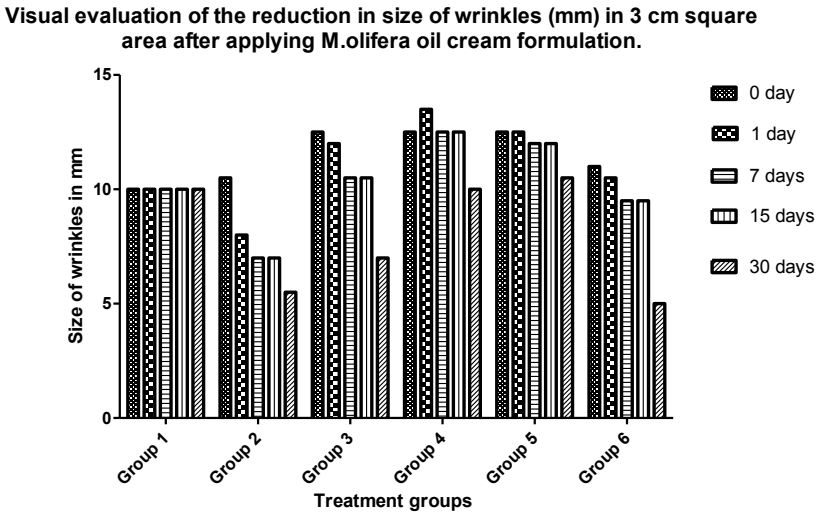
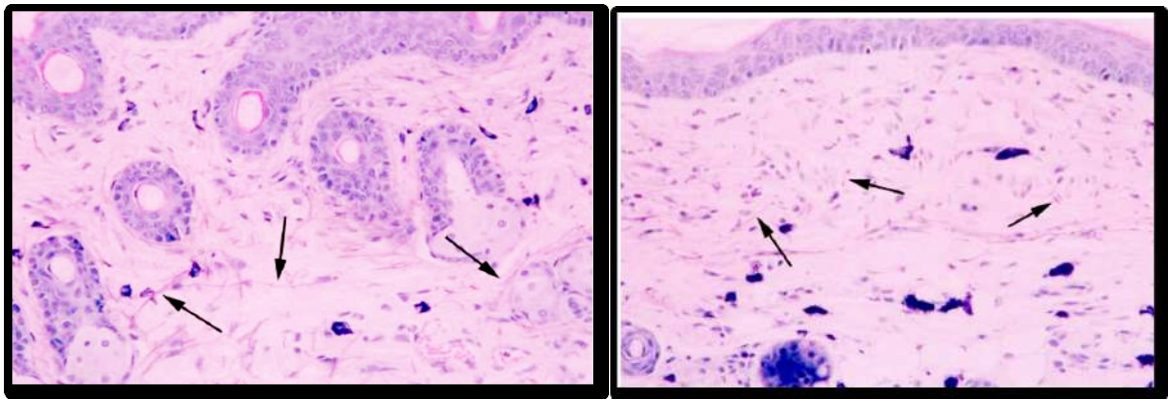
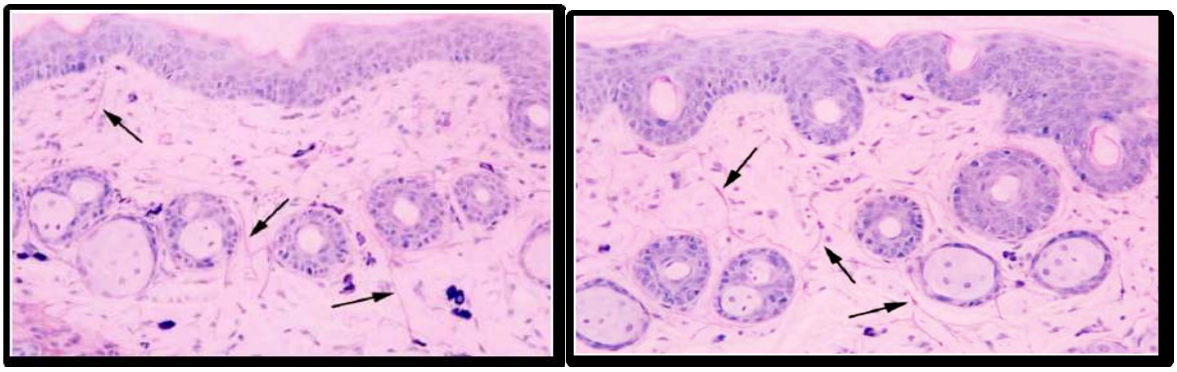


Figure 10-b: Shows analysis of the visual evaluation of the reduction in size of wrinkles (mm) in 3 cm square area after applying *M.olifera* oil cream formulation.



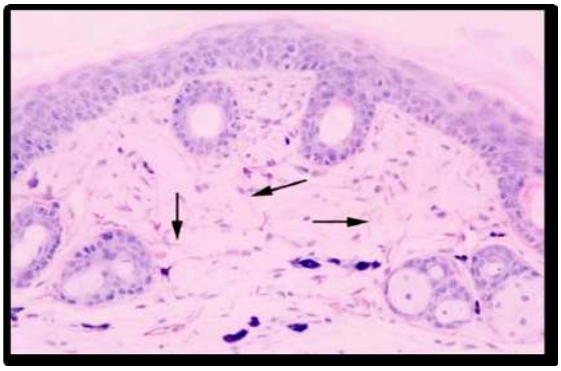
Group 1: normal group

Group 2: The elastic fiber was definitely decreased by UV irradiation



Group: 3

Group 4: The elastic fiber was definitely decreased



Group: 5

Figure 11: Histological evaluation of mouse skins

IV. Summary and conclusion:

In the present study, we prepared *Moringa oleifera* oil based cream and nano-emulsion and system. The prepared cream and nano-emulsion system were characterized and anti-wrinkle effect *in vivo* was evaluated.

The soxhlet extraction yielded 32.11% oil extract which is higher when compared ot manual extraction. The *Moringa oleifera* Oil in water (O/W) emulsion-based cream was formulated using various concentration of emulsifier. The formulated cream was evaluated for various *in vitro* physical evaluation tests in room and accelerated temperature upto 20 days and tested at various intervals. The formulation 3 showed pH 6.0-6.3 in room temperature and pH 5.8 - 6.0 at 40°C. The formulated cream showed good homogeneity, Spreadibility, No change in colour and was Emollient, Non greasy and easy to remove. The *Moringa oleifera* seed oil based cream showed no redness, edema, inflammation and irritation during irritancy studies. The formulation F3 was safe to use for skin and selected for further *in vivo* study.

Moringa oleifera nano-emulsion(MONE) was prepared using various concentrations of moringa oil and all the formulations were characterized. All formulations were analyzed directly after production the critical parameters such as particle size, PDI and Zeta potentials were determined. The mean particle diameter of MONE 3 was found to be 51.0 nm. The poly dispersity index of optimized formulation MONE 3 was 0.22 and the Zeta potential 43mV. The MONE 3 nano-emulsion droplets were showed discrete, uniform and spherical with a smooth surface.

The hairless mouse (SKH-1) was used for the *in vivo* anti-wrinkle efficiency study. The skin wrinkling was induced by UVB irradiation at 365 nm. The anti-wrinkle score was observed visually by taking photographs before treatment. After 30days treatment with the formulations and the standard, the wrinkle score, number of wrinkles and size of wrinkles was observed microscopically and also in photographs and the results were tabulated. The histological examination of skin specimens were stained by the Pinkus' acid orcein-Giemsa staining method. The *Moringa oleifera* Oil nano-emulsion (MONE 3) showed better protective effect compared to *M. oleifera* Oil o/w cream Formulation-3. The possible mechanism of the anti-wrinkle effect of *Moringa oleifera* Oil based cream formulations was hypothesized as follows. The nano emulsion delivered to the viable dermal layer where the antioxidant constituents has to be delivered and the delivery was promoted by the hydrating effect on the skin surface. However, due to its hydro phobic nature of *M. oleifera* formulations were deposited mainly into the viable dermal layer. The *M. oleifera* Oil formulations exerted an anti-oxidative effect, which protected against the loss of elastin, ultimately resulting in the anti-wrinkle effects.

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