Open Access Journal
International Journal of Medical Research and Pharmaceutical Sciences
Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

FORMULATING A THERAPEUTIC CREAM FOR BURN INJURY USING CYANOBACTERIAL EXTRACTS AND GOLD NANOPARTICLES

Seok Gyu Han*[1], Jun Myung Song[2] and Jongbin Lee[3]
*[1,2 & 3]Provert Research Laboratory Englewood Cliffs, NJ 07632

Keywords:
Antibacterial Strength, Burn Cream, Gold Nanoparticle, Skin Injury, Spirulina Platensis.

Abstract
The field of medication has advanced in a rapid way. Most attention has been directed towards the treatment of global scale diseases like cancer, diabetes, and AIDS. However, the treatment of non-transmittant illnesses, such as burn injuries, has not received enough attention. Burn injury is one of the leading causes of morbidity in the world. Globally, thousands suffer from long-term consequences due to the lack of effective first-aid treatment burn injury. Many commercial burn creams available today have been found to be ineffective in providing the needs that must be fulfilled to treat a burn wound effectively.
This research of formulating a therapeutic cream has been motivated as a way to alleviate the pains of burn patients. The cream has been formulated, considering on the three essential elements: antibacterial strength, moisturizing capability, blister reduction ability, and anesthetic effect. *Spirulina Plantensis* extract and gold nanoparticles have been synersized for maximum antibacterial strength. Glycerine for moisturization and sodium dodecyl sulfate for blister reduction were enforced. Lidocaine has been added to provide anesthetic effect to the cream. Various tests have been conducted and verified the effectiveness of our burn injury cream.

INTRODUCTION

According to the World Health Organization Publication, approximately 300,000 deaths have been caused by burn injuries. Most of the deaths have occurred in undeveloped regions of the world, where the necessary medical attention and treatment for burn injuries lack the most [1]. A burn injury is usually caused by a direct contact with the skin and the fire. In the process, heat is transferred from flame to the skin, damaging the epidermal layer of the skin and thus generating blisters [2]. In worse cases, heat may even damage skin layers that are below the epidermis, which are namely called dermis and hypodermis. The destruction of these tissue cells leaves excruciating pain to the victim. In most cases, victims with severe burns suffer long-term consequences, such as the disfigurement, scars, recurrent infections, and the loss of mobility. In addition to physical consequences, victims also tend to experience emotional stress due to the burn injury [3-5].

In cases of burn injuries, especially for the severe ones, it is crucial that a first-aid treatment is implemented before receiving the adequate care from healthcare professionals. Providing immediate attention to the wound is an effective way to prevent short and long term effects of a burn. There are various first-aid treatments commercially available in the public [6]. For example, Bacitracin is one of the most commonly used treatments for burn injuries. However, it has been reported to cause skin troubles, allergic reactions, and delayed healing to some people [7]. In addition, it has been reviewed that Bacitracin fails to remove blisters properly [8].

Three key features must be present in an effective burn cream: the ability to resist bacterial infection, to improve the condition of the skin, and to relieve pain [9]. Antibacterial resistance should be one of the strongest traits of the cream. It is often bacterial infection that causes exacerbates burn wounds and delays healing. As a strategy of increasing the antibacterial strength of the burn cream, gold nanoparticles and Spirulina extract were added to the cream.

Gold nanoparticle(AuNP) is a colloidal suspension of sub micrometer-sized particles of gold in water. The product has a color of deep red and blue depending on the size of its particles [10]. AuNP has been described in many substances as a powerful antibacterial substance [11-13].

Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

Along with AuNP, Spirulina extract has also been used to formulate the cream. Ethanol content from the *Spirulina P latensis* (*S. Platensis*) extract increases the antibacterial strength of the burn cream. The capability of the spirulina e xtract not only promotes its antibacterial effects [14, 15], but it also contains anti-oxidants that can enhance the imm unity of the body and the health of skin cells [16]. Antioxidants remove destructive non-electron paired oxygen spec ies in the injured area as well as other parts of the body, thus improving the immunity [17].

As a way to strengthen anesthetic ability to the burn cream, lidocaine has been added to our burn cream. The substan ce is able to reduce pain by numbing the damaged area, thus acting as an anesthetic [18, 19].

Furthermore, glycerine and sodium dodecyl sulfate (SDS) were blended for improving the condition of the skin. Gly cerine increases the ability of the skin to retain water. Moisturization facilitates skin tissues to become smooth and fl exible [20], allowing burn wounds to recover quickly while minimizing the scar of the wound. On the other hand, S DS was used to remove blister of the burn wound. SDS is a penetration enhancer [21, 22], which allows the surface of the blister to become soft and allow fluid to be removed through osmosis. Regarding these benefits, the addition of glycerin and SDS is an appropriate choice in formulating a burn cream specifically for burn wounds.

At every step of formulating procedures, the quality of the intermediate creams has also been tested with various met hods.The indirect viscosity test, microscopic observation, and centrifugal test have been conducted to determine the optimal conditions for the final product. These tests were deemed to be significant in developing a stable burn cream with high effectiveness.

EXPERIMENTAL METHODS

Reagents and Materials

Spirulina powder was purchased from ZNaturalFoods(West Palm Beach, FL). Lidocaine was obtained from Sigma-Aldrich (St. Louis, MO). Dodecyl sodium sulfate was purchased from Carolina Biological Supply Company (Burlin gton, NC). Olive oil was the product of Salov North America Corp (Lyndhurst, NJ). Emulsifying wax was from Plan t Guru (Plainfield NJ). Non-pathogenic Escherichia coli was used for the general antibacterial effect of the combine d cream (Carolina Biological Supply Company, Burlington, NC). Blocks of acorn jelly were purchased from local A sian market (Fort Lee, NJ). Hematocrit centrifuge Autocrit (Alert Scientific Inc, East Hartford, CT) was used for t he cream base (CB)'s stability test [23]. The microscope with 2000x magnification install- ed with a high density dig ital camera (Amscope, Irvine, CA). The Soxhlet Apparatus was manufactured from Suzhou (China).

Ethanol-Based Spirulina Extraction

Dried Spirulina of 15 g was pulverized and poured into the sample chamber of the soxhlet apparatus as established b y Khan et al. [24] and Mala et al. [25]. in 200 mL ethanol, and distilled for 24 hours. The final product of spirulina e xtract was kept at 4 °C in the refrigerator until used.

Cream Base Emulsion Formulation

Two beakers of 500 mL were prepared: one was filled with 40 mL distilled water and another with the mixture of 18 mL olive oil and varying amounts of emulsifying wax (4g, 8g, 12g). And, the two beakers were placed in a water ba th which was put on top of a burner. The contents of the beakers were heated up to 70°C degrees, and the oil phase mixture was stirred. Then, the warm water was poured into the beaker with olive oil and emulsifying wax. The cont ents of the beaker were stirred using JJ-1 Accurate Electric Stirrer under 500~800 rotations per minute (RPM) for 12 0 minutes.

Cream Base Stability

Various tests were carried out to verify the stability of each type emulsion as follows.

Viscosity of Cream Base

The viscosity of the cream base(CB) determines the stability of the emulsion. After stirring the mixture for 120 min utes, a 2.5 cm marked plastic tube was dipped into the emulsion. Then, the plastic tube was weighed on a milligram

Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

scale. And, the amount of cream base stuck on the surface of the plastic tube was evaluated.

Microscopic Observation

An ideal emulsion contains stable oil droplets of 10-30 um dispersed uniformly throughout. A drop of CB from each container of 4, 8, and 12g wax emulsion was smeared onto the surface of slide glasses. The CB was then stained wit h Eosin Y to enhance the visual contrast. The microscopic observation was conducted 48 hours repeatedly.

Centrifugal Breakage Test

The stability of the cream was examined through a hematocrit centrifugal test with Model Autocrit Ultra 3. Cream s amples from each container of 4g, 8g, and 12g wax emulsion was pulled into hematocrit capillaries using 1 mL tuber culin syringe. Then, one end of the capillary was blocked with clay to prevent the samples from spilling out. Then, each sample was placed on a circular cartridge of the centrifuge. The centrifuge was then run at 20,000 rpm for one minute. After the process, the length of liquid and emulsion layers in the capillary were measured.

Combining AuNP with Cream Base

After the formulation of the CB, AuNP solution was added and mixed thoroughly. The 4g, 8g, and 12g CB were divi ded into three 200 mL beakers. Then, 15 g of each cream was weighed out on the Petri dishes and poured with the 1 0 mL, 15 mL, and 20 mL of AuNP solution. All prepared cream with different amount of emulsifying wax, and diff erent volume of the AuNP. The ingredients were well blended manually and stored in the refrigerator for the subseq uent step.

Antibacterial Effect Observation

E. Coli has been used to determine the antibacterial effect. The first set of *E. Coli* was cultivated 48 hours in advanc e for its immediate usage, while circular filter-paper disks (7 mm diameter) were prepared to be soaked in burn crea m samples that contained various amount of AuNP (10, 15, 20mL). Two sets of cream samples each containing 4g, 8g, and 12g of wax were prepared. One set of sample contained the spirulina extract, while the another did not. The n, three agar plates were prepared with the E. Coli culture for the 4, 8, 12 g wax CB, respectively. The paper disks w ere then soaked into various cream mixtures and put on to the agar plate.

After the incubation at 37 degree, the radius of zone of inhibition was measured as the indicator of the antibacterial e ffect.

After inoculation, the 7mm paper disks were soaked with creams that mixed with varying amount of AuNP, 10, 15, 20 mL. This set of sample does not contain the spirulina extract.E.Coli was cultured on the agar plate with three crea m-soaked paper disks for the antibacterial test. Each plate designed for the creams of 4, 8, 12 g wax, and in each plat e, with 10, 15, and 20 mL AuNP cream. This set of sample contains the spirulina extract.

Moisturization and Blister Dry-up Effects

This test has been conducted to examine the effectiveness of glycerine and SDS in retaining moisture of the skin an d removing blisters. An acorn jelly was cut into various cubes, and were coated with various types of cream bases. 2 g glycerin was added to cream bases containing 4, 8, 12g of emulsion wax. The following are the various cream base s used: CB 4g, 8g, 12g, 4g+glycerin, 8g+glycerin, 12g+glycerin. One sample jelly was left uncoated. Fig. 4 shows t he complete processes of the moisturization test, and the experiment conditions were recorded on the Petri dishes. T he cubed jelly weighed at 12, 36, and 60 hour post-application.

Seven acorn jelly cubes were prepared for moisturization test. Glycerin was introduced as a way to improve moistu rization. Acorn jellies on the second row with the cream were the samples of the experiments. The acorn jelly witho ut any cream was used as control on the third row.

As for blister dry-up effects, SDS was used to enhance the permeability of the skin. Cream containing SDS of 0%, 0. 5%, and 1% was carefully coated on to the skin of *L. terrestris*. Then, the difference of body weight before and after an hour of application was measured.

Open Access Journal
International Journal of Medical Research and Pharmaceutical Sciences
Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

Anesthetic Effect Test Using Lidocaine

In a burn treatment, anesthetic effect is one of the important elements that must be present. Lidocaine of 0.2, 0.4 g w as added to creams containing 4, 8, and 12g emulsifying wax. Creams containing 0.2 or 0.4 lidocaine were applied t o five *L. Terrestris*. Their reaction and behavior toward physical touch and light were recorded as our grading syste m.After lidocaine added, the cream mixture was applied to three L. Terrestris. And, their behavioral and neurologic al responses were scored at every 20 minutes.

RESULTS AND DISCUSSION

Relative Viscosity of Cream Base

Figure 1 shows an approximate linear relationship between the amount of wax and the weight of emulsion stuck on t he plastic tube. The emulsion containing 12g of wax was the highest viscosity of the three emulsions. A viscous emu lsion often has greater stability and balance between oil and water than loose emulsions. As a result, the data below s hows that the emulsion with 12g of wax is the most appropriate cream base to use.

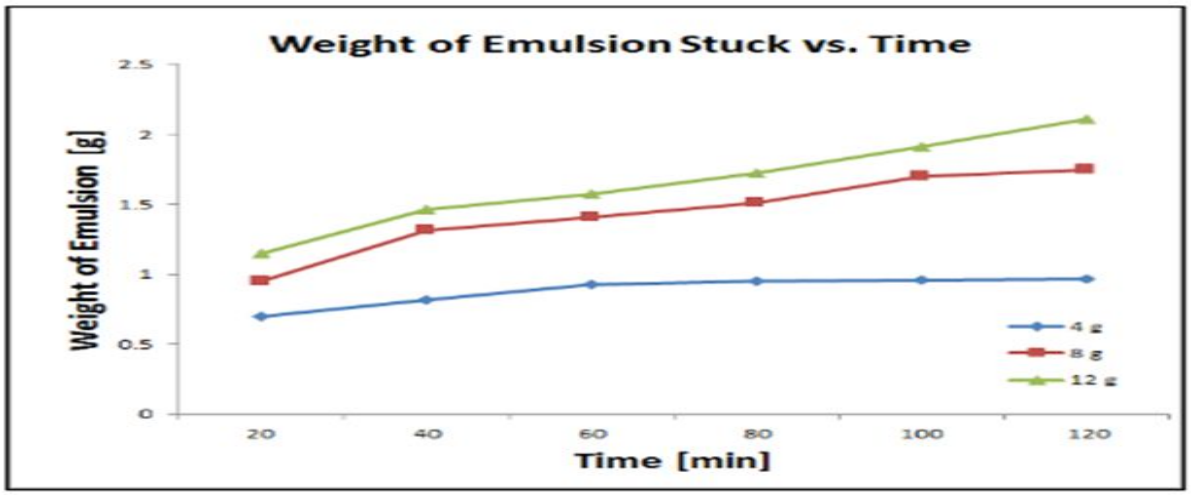

Fig. 1 The graph presents the relative viscosity of the cream bases with the relationship between emulsifying wax on the time of stirring. The amount of emulsifying wax and stirring time are directly related (mean, N=4).

Microscopic Observation

According to observation of droplets present in the cream base, the size and compositions of droplets of every emuls ions were different. In the 4g-wax cream, large, numerous droplets were observed. Some of the droplets seemed to c oalesce with another. In 8g-wax cream, droplets were slightly smaller than the one of the 4g-wax cream. In 12g wax cream, the droplets were significantly smaller than those of other two creams. In addition, most of them were not co alesced. These qualities of the 12g cream shows that oil and water are mixed very thoroughly, thus enhancing the m orphology and the stability of the cream. Microscope equipped with Amscope camera offered the visual images of t he CB. The location indicator pin was used for taking pictures at the same location.

Centrifugal Test for the Stability

The quality of the mixture can be well determined by the ratio between the liquid and the emulsion layer. For the cre am containing 4g of wax, the ratio was almost at a 1:1 ratio, showing that the emulsion was relatively weak. For 8g of wax, the length of emulsion layer was slightly greater than the liquid. For 12g of wax, the capillary tube was almo st completely filled with emulsion layer. This showed that the balance and the stability of oil-water mixture was the greatest for 12 g of wax emulsion. As seen in Fig. 3, the layer of cream layer was proportionally increased with resp ect to the amount of the emulsifying wax.

Open Access Journal
International Journal of Medical Research and Pharmaceutical Sciences
Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

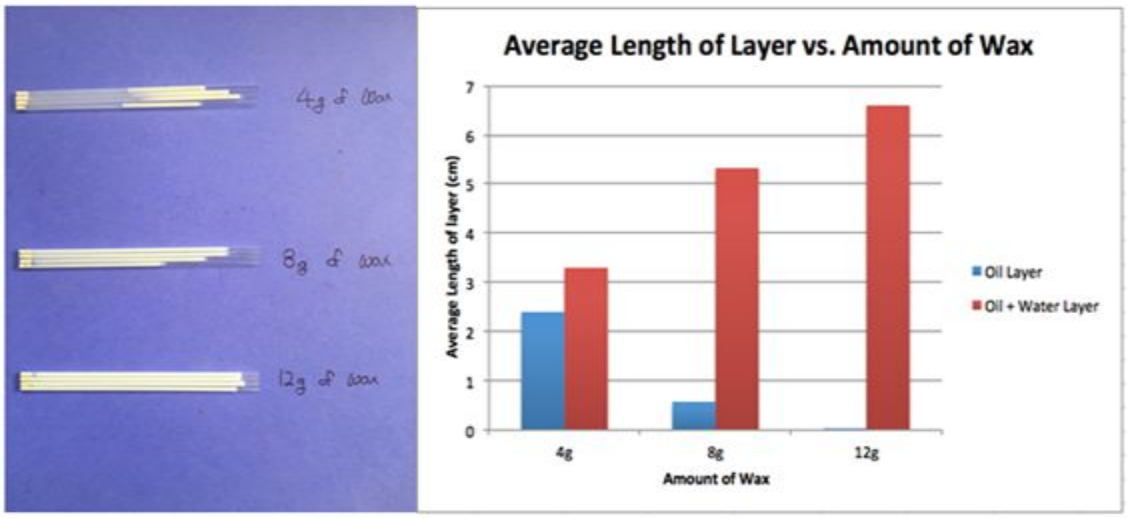

Fig. 2. The left image presents the capillary views after centrifugal stress. Right image shows relationship betwee n layer ratio and amount of emulsion wax.

Antibacterial Effect

The data provided in Fig. 4 was given by mean values (N=4). For the three dishes containing 4, 8, 12g cream witho ut spirulina extract, the inhibition distance was measured to be the same at 0.21 cm. In contrast, for the cream with t he addition of spirulina extract, the inhibition radius significantly increased up to 0.35 cm. Based on the data, it was found that there is a relationship between the addition of spirulina extract and antibacterial effect. However, in contr ast, it was found that there is no relationship between the amount of AuNP and the antibacterial effect. Various amo ung of AuNP were used, yet the results were the same. Based on this data, it was found that AuNP only has a slight i mpact on the antibacterial effect.

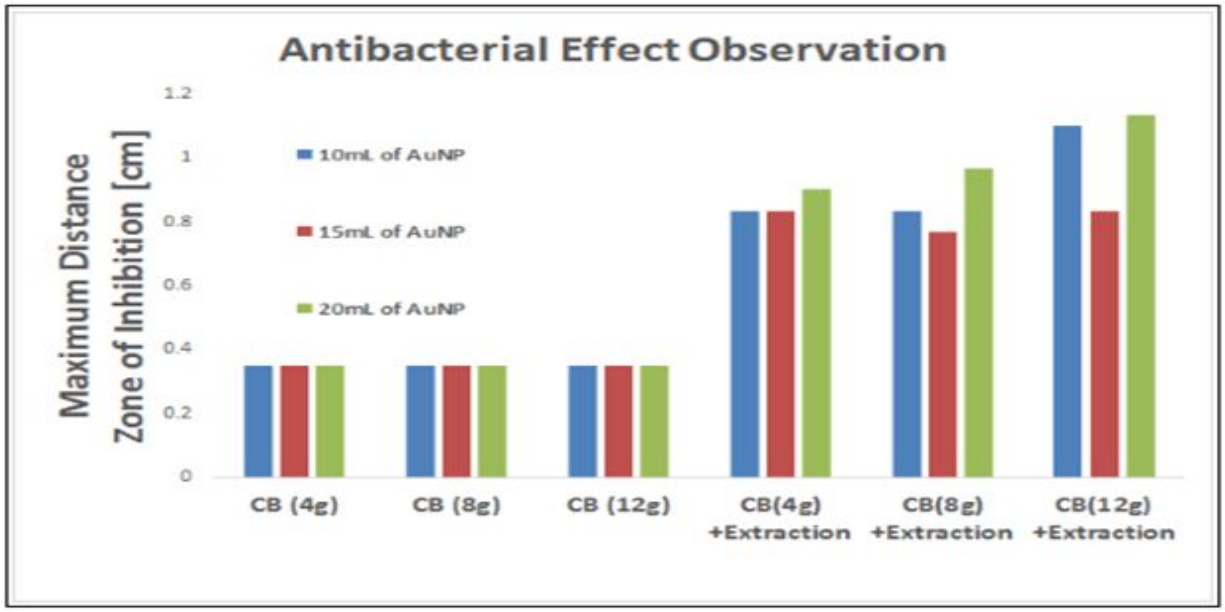

Fig. 3. The data of the antibacterial effect were reported, and the data showed differently by the addition of spirul ina extract. The spirulina extract seemed to give a significant impact on the antibacterial effect.

Open Access Journal
International Journal of Medical Research and Pharmaceutical Sciences
Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

Moisturization and Blister Dry-up Effects

The acorn jelly blocks were measured at every 12, 36, and 60 hours post-cream application. According to the observ ation, the weight of the jelly block without cream changed the fastest, showing no ability to retain moisture. The on es covered with the emulsion cream were able to maintain their initial weight because the cream continued add mois ture to the jelly. Overall, this observation showed us that the addition of glycerin plays a crucial role in providing mo isturization to the jelly. Among all groups of this study, the mean values showed that CB 4g + Glycerin combination showed the best water retention ability up to 60 hours. The result can be seen in Fig. 4.

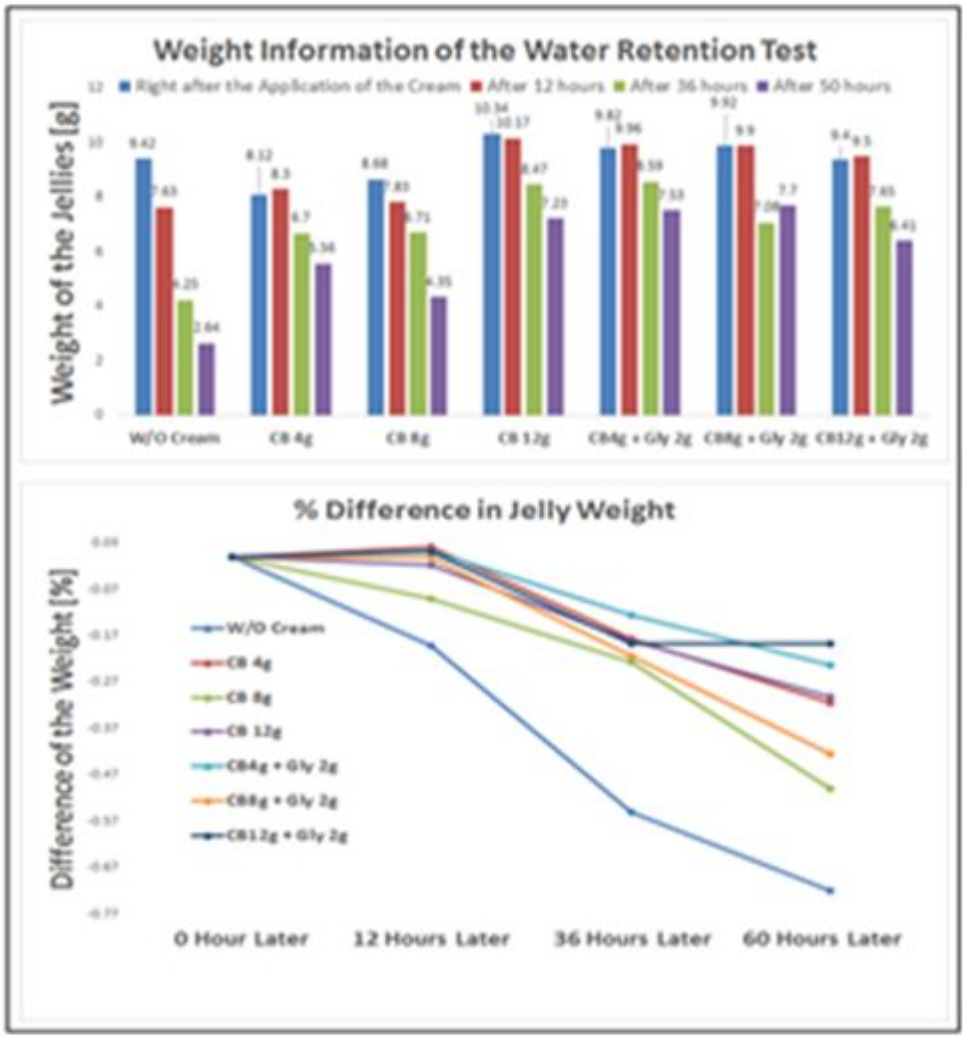

Fig.4. These two data show the results of the moisturization test. Top bar graphs shows the actual weight, and bot tom line graph shows the decreased percentage of the weight by the time.

As for SDS, the result showed that the body weight reduced the most for the *L. terrestris* that were coated with crea m containing the most SDS concentration (1%). This shows that SDS can make the layer of skin soft enough for the fluid in the blister to be penetrated outside the surface as seen in Fig. 11. The 12g was cream with spirulina extract a nd AuNP was blended with 0, 0.5, 1.0% SDS, respectively and applied evenly to the skin of L. terrestris skin. Their body weight was measured after 1 hour.

Open Access Journal
International Journal of Medical Research and Pharmaceutical Sciences
Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

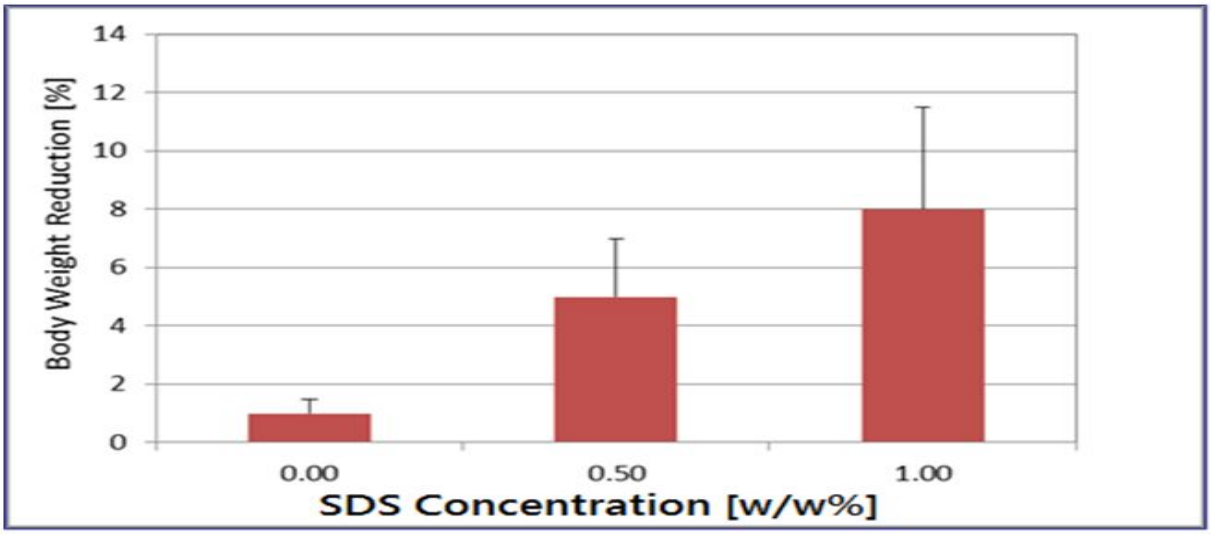

Fig.5 Blister Reduction was proportional to the SDS concentration.

Anesthetic Effect of the Cream

A behavioral scoring system of *L. Terrestris* was used to find the effectiveness of adding lidocaine to the cream. When the organisms were fully awake and active, a score of 5 was recorded. In contrast, when they were fully anesthetized and immobile, a score of 1 was recorded. According to the data, there was a direct relationship between the amount of lidocaine with the movement of the *L. Terrestris*. At 2%, the behavior score was, on average, higher than the behavior score of 4% lidocaine. This shows that lidocaine effectively provides an anesthetic effect to the cream. Both 2% and 4% concentrations did not show any pharmacological or physical side-effects after the application.

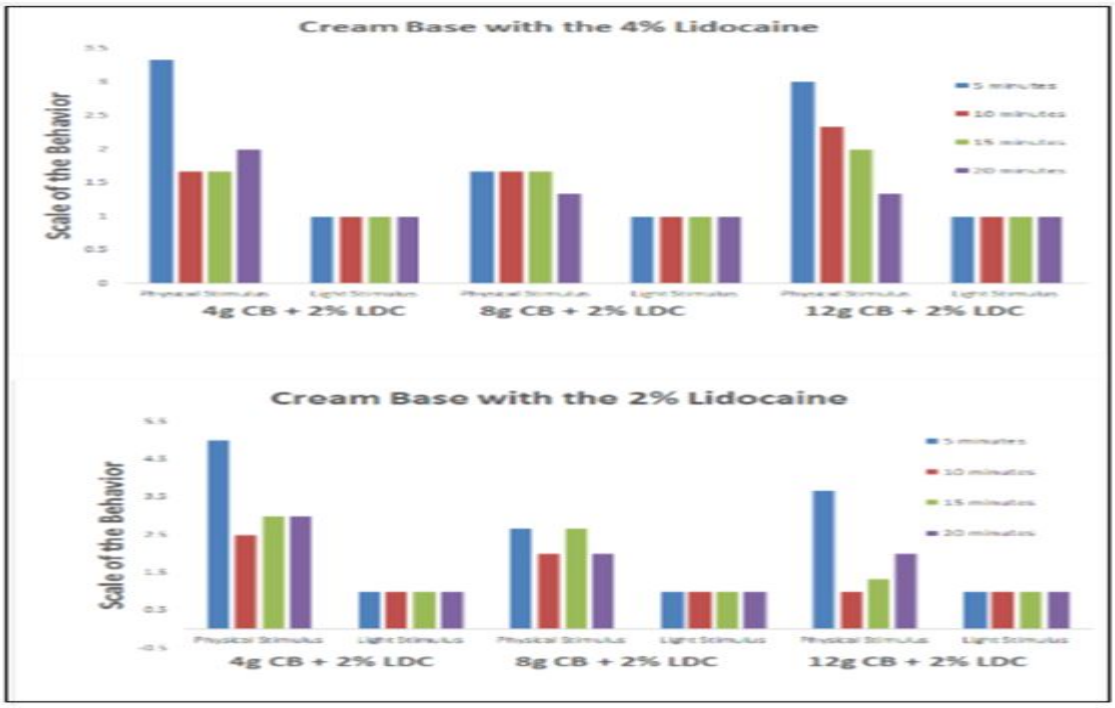

Fig.6. The graphs presents the anesthetic effect of the cream. The 8g-wax cream showed the fastest pharmacological effect in both concentrations.

Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

CONCLUSIONS

A new burn injury cream has been formulated on the cream base with the addition of spirulina extracts, AuNP sodium dodecyl sulfate, glycerin, and lidocaine. Various tests confirmed the hypothesis of this research that the formulated burn injury cream satisfies the three essential characteristics of an effective burn treatment, It should provide antibacterial effect, improvement of the skin, and anesthetic effect to the wound. There are some limitations to this experiment. The burn cream has not been tested on a human skin or on actual burn wound. However, the tests conducted in the project provide enough information and data to validate the hypothesis of the experiment and the effectiveness of the formulated burn cream.

REFERENCES

1. *Charles Mock, Michael Peck, et al. "A WHO Plan for Burn Prevention and Care", World Health Organization Document Production Services, Geneva, Switzerland, 2008.*
2. *Xu, F., Wang, P F, Lin, M., Lu, T. J., NG, E.Y.K., "Quantification and the Underlying Mechanism of Skin Thermal Damage: A Review", Journal of Mechanics in Medicine and Biology, vol. 10, no.3, 373-400, 2010.*
3. *American Red Cross, "Responding to Emergencies; Comprehensive First Aid/CPR/AED [Internet] http://www.instructorscorner.org/Textbook/par794omgqsc30adj9nm2hol6x1/656138_RTE_Textbook_FinalPDF_Digi2014.pdf, 2014.*
4. *Hunter John, Savin John, and Dahl Mark, "Clinical Dermatology", Blackwell Science Ltd Publishing Company, 2002.*
5. *Gretchen J. Summer, Kathleen A. Puntillo, Christine Miaskowski, Paul G. Green and Jon D. Levine, "Burn Injury Pain: The Continuing Challenge", The Journal of Pain, vol 8, no. 7, pp. 533-548, 2007.*
6. *John Greenwood, Sheila Kavanagh, "First aid and emergency management of adult burns; 2011 Practice Guidelines; Burn Unit Direct Line", Government of South Australia: Internet:http://www.rah.sa.gov.au /burns/ down loads /First-aid-book2011for- Web.pdf, 2011.*
7. *Barajas-Nava, L.A., Lopez-Alcalde, J., Roque I Figuls, M., Sola, I, Bonfill Cosp, X., "Antibiotic prophylaxis for preventing burn wound infection (Review)", The Cochrance Collaboration. Published by John Wiley & Sons, Ltd, The Cochrane Library, Issue 6, 2013.*
8. *Charles Cole, John Gazewood, "Diagnosis and Treatment of Impetigo", Am Fam Physician, vol. 75, pp. 859-64, 2007.*
9. *Siobhan Connolly,"Clinical practice guide- lines: Burn patient management", ACI Statewide Burn Injury Service, Agency for Clinical Innovation, 2012, Internet: http://www.aci.health.nsw.gov.au/data/assets/pdf_file/0019/162631/Clinical_Practice_Guidelines_2012.pdf, 2012.*
10. *Khalida S. Merza, Hadi D. Al-Attabi, Zaid M. Abbas, Hashim A. Yusr, "Comparative study on methods for preparation of gold nanoparticles, Green and Sustainable Chemistry, vol. 2, pp. 26-28, 2012.*
11. *Shahzadi Shamaila, Noshin Zafar, Saira Riaz, Rehana Sharif, Jawad Nazir and Shahzad Naseem, "Gold Nanoparticles: An efficient antimicrobial agent against enteric bacterial human pathogen", Nanomaterials, vol. 6, no. 71, pp.1-10, 2016.*
12. *Yan Zhou, Ying Kong, Subrata Kundu, Jeffrey D. Cirillo and Hong Liang, "Antibacterial activities of gold and silver nanoparticles against Escherichia coli and bacillus Calmette-Guerin", Journal of Nanobiotechnology, vol. 10, no. 19, pp.2-9, 2012.*
13. *Thanaa Majied Al-Nori, "Antibacterial activity of sivler and gold nanoparticles against Streptococcus, Staphylococcus aureus and E. coli', Al-Mustansiriya J. Science, vol. 23, no. 3, pp. 45-54, 2012.*
14. *Sudha S.S., Karthic R., Rengaramanujam J., Athulya, "Antimicrobial Acitivity of Spirulina platensis and Aphanothece sp. on selected clinical bacterial isolates and its antioxidant activity", Sluth As. J. Biology Science, vol. 1, no. 2, pp. 87-98, 2011.*
15. *Vinay Kumar, Bhatnager A. K., and Srivastava, J. N., "Antibacterial activity of crude extracts of Spiruina platensis and its structural elucidation of bioactive compound", J. Medicinal Plants Research, vol. 5, no. 32, pp. 7043-7048, 2011.*
16. *Alexsandar Godic, Borut Poljsak, Metka Adamic, and Raja Dahmane, "The role of antioxidants in skin cancer prevention and treatment", Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity, vol 2014, Article ID 860479, pp. 1-7, 2014.*
17. *Lobo, V., Patil A., Phatak A., and Chandra N., "Free radicals, antioxidants and functional foods: Impact*

Volume 4 (Issue 3): March 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.376416 Impact Factor- 3.109

on human health", Pharmacologn Rev. vol. 4, no. 8, pp. 118-126, 2010.

18. *Philip Grady, Nathaniel Clark, John Lenahan, Christopher Oudeke 가, Robert Hawkins, Greg Nezat, Joseph E. Pellegrini, "Effect of intraoperative intravenous lidocaine on postoperative pain and return of bowel function after laparoscopic abdominal gynecologic procedures, " AANA Journal, vol. 80, no. 4, pp. 284-290, 2012.*
19. *Jianming Liu and Xin Lv, "The pharmacokinetics and pharmacodynamics of lidocaine-loaded biodegradable poly9lactic-co- glycolic acid) microsphere", Int. J. Mol. Sci., vol. 15, pp.17469-17477, 2014.*
20. *Anthony Simon F., Eric S. Abrutyn, and Zoe D. Draelos, "Ability of moisturizers to reduce dry skin and irritation and to prevent their return", J. Cosmet. Sci., vol. 56, pp. 427-444, 2005.*
21. *Anushree Pandey, Ashu Mittal, Nitesh Chauhan and Sanjar Alam, "Role of surfactants as penetration enhancer in transdermal drug delivery system", J. Mol. Pharm. Org. Process Res, vol. 2, issue 2, pp. 2-10, 2014.*
22. *Inayat Bashir Pathan, Mallikarjuna Setty C., "Chemical penetration enhancers for transdermal drug delivery system", Tropical J. of Pharmaceutical Research, vol. 8, no. 2, pp. 173-179. 2009.*
23. *Edward R. Garrett, "Prediction and evaluation of emulsion stability with ultracentrifugal stress", J. Soc. Cosmet. Chem., vol. 21, pp. 393-414, 1970.*
24. *Khan Barkat Ali, Akhtar Naveed, Rasul Akhtar, Mahmood Tariq, Khan Haji Muhammad Shoaib, Shahip-Uz-Zaman, Muhammad Iqbal and Ghulam Murtaza, "Investigation of the effects of extraction solvent/technique on the antioxidant activity of Cassia fistula L.", Journal of Medicinal Plants Research, vol. 6, no. 3, pp. 500-503, 2012.*
25. *Mala R., Sarojini M., Saravanababu S., and Umadevi G., "Screening for antimicrobial activity of crude extracts of spirulina plantensis", Journal of Cell and Tissue Research, vol. 9, no. 3, pp. 1951-1955, 2009.*