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A Novel Action of Silver Nanoparticles for Skin Repair

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Abstract

The Silver nanoparticles have been widely used as new potent antimicrobial agents in cosmetic and hygienic products, as well as in new medical devices. Silver compounds have also been used for centuries for healing purposes because of the broad spectrum antibiotic, and have been proven to be effective in both, retarding and preventing bacterial infections and healing wounds. In this study the evaluation of healing properties of Silver nanoparticles has done by using hydrophilic ointment containing nanosilver. This ointment was prepared and used for anti-inflammatory and wound healing study. In the study of silver nanoparticls effect on anti-inflammatory study showed reduces edema with 91% (inhibition percentages) as compared to standard which showed 81% (inhibition percentages). In further study of Silver nanoparticle had shown significant therapeutic activity in excision wound study. In this study the mean percentage closure of wound for AgNP used in 1% of concentration had shown better result with 95.28% in comparison with standard which was around 93.71%. This was further confirmed by the collagen and epithelization studies. There was significant difference between the entire group and the 1% AgNP ointment was found to be highly effective. As the collagen synthesis is the first step due to the formation of precursor polypepetide such as lysine and proline residues. The remolding of scar tissue occurs due to the fast inter molecular and intra molecular cross linking of collagen. Collagen not only confers strength and integrity to the tissue matrix but also plays an important role in hemostasis and epithelialization at alater phase of wound healing. With this study we can state that the use of Silver nanoparticles in cosmetic formulation can help in reducing wrinkles and increase the firmness of the skin as well as it can be used in anti-acne preparation efficiently.

Keywords

Silver Nanoparticle, Wound Healing, Cosmetic, Acne, Antiaging, Skin Rejuvenation, Collagen Booster

1. Introduction

Today nanotechnology is overcoming the barrier of commonly used therapies for treating acute and chornic wounds. The cosmetic products where the nano silver can be used are, for example, moisturizer using nanosilver as preservative; face wash as antibacterial, after shave lotion as anti-inflammatory and wound healing. Cosmetics industry is always looking to improve the properties of its products and hence is making more and more use of the developments in nanotechnology [1]. There are no evidence-based medical uses for ingested colloidal silver at present. Indeed, the U.S. National Center for Complimentary and Alternative Medicine has issued an advisory indicating that the marketing claims made about colloidal silver are scientifically unsupported. Despite this, interest in the clinical use of silver has been rekindled due to the availability of silver nanoparticles (AgNPs). As the diameters of AgNPs are generally smaller than 100nm and contain 20–15000 silver atoms. Exposing cells or tissue to AgNPs, the active surface of AgNPs would be significantly large compared to silver compounds, and there by exhibiting remarkably unusual physicochemical properties and biological activities. Despite the fact that AgNPs have been increasingly applied in the cosmetic and biomedical or pharmacological fields, relatively little

research has been done in cosmetic [2]. The herapeutic use of nanotechnology based drugs in the near feature should be widely spread because of its biodegradable and nontoxic properties [3].

As the skin is the largest external organ in the human body. The composition of the skin is intricate involving multi faceted layers responsible for various functions. There are two major components of the skin: the epidermis and the dermis. The epidermis covered with keratin is responsible for the physical biochemical/chemical and adaptive immunological barrier functions. Skin damage is such a common occurrences in daily life that its effects are passed over as negligible, however in reality it poses a threat to the security of the entire body. The human body can experience a variety of injuries, including penetration, burns and blunt trauma. Some of these forms of damage can turn into chornic wounds, which cause long term agony and complication in many patients, as a result of the inability of the body to properly heal itself [4]. Impaired skin integrity leads to the development of a wound that could involve different tissues from the epidermis to deeper layers such as muscles [5].

Silver compounds have been used for centuries for healing purposes because of the broad spectrum antibiotic. Since 1968 it has been found in the form of ointments and cream which was used immensely to prevent infection. On the other hand silver nanoparticles are able to overcome the antibiotic resistant bacteria. Due to its anti-inflammatory effects and neovascularization it can accelerate the wound healing process efficiently [6, 7]. Along with it Silver based medical products have been proven to be effective both in retarding and preventing bacterial infections and healing wounds [8]. Thus the study focuses on the effects of AgNP on wound healing and more precisely tissue rearrangement due to its potential to modulate the collagen deposition which confirms better fibril aliments in repaired skin. In the study reported herein, better cosmetic appearance was observed in animal treated with silver nanoparticles. In terms of wound healing, various parameters give the information which plays an important role in tissue fibrosis and post injure scarring. In wound care management the prevention of wound infection and the retention of an appropriate level of moisture are two major challenges. AgNP promoted wound healing ability as the skin treated with AgNP group showed less adverse changes enhanced angiogenesis accelerated re-epithelialization and quick complete healing compared to all other group.

Silver particles have antibacterial, anti-inflammatory and wound healing properties. These are used very sparingly in cosmetic industry, and hence its complete potential as an active ingredient in cosmetic formulations is underutilized. This is also due to lack of supporting research data available. This makes the silver, the best case to study its application in cosmetic formulations. The nanosilver will certainly show improved efficacy over its bulk, as the nanoparticles - as a rule exhibit, vast surface area owing to their size.

2. Anti Inflammatory Study

2.1. Experimental Animals

Male Albino rats weighing 200-250 gm is used for animal studies. The animals are grouped in cage and maintained under standard laboratory condition (temp 25±2°C) and relative humidity (50±5%) with dark and light cycle (14/10hr). The experimental protocol was approved by Institutional animal ethical committee 1426/PO/Re/S/11/CPCSEA; Date: 1/08/2016. The rats were acclimatized to laboratory condition for 14 days before commencement of experiment. The animals were provided with commercial food.

2.2. Chemicals.

Carrageenan, Standard drug Diclofenac sodium.

2.3. Experimental Design

Acute inflammation is provided by injection of 0.1ml of 1% Carrageenanin to the subplantar surface of rat hind paw.

Group 1 (control): Rats were received 0.1ml of 1% Carrageenan.

Group 2 (standard): Rats were received Diclofenac (40mg/kg/ip).

Group 3 (Test1): 1% ointment sample and 0.1ml Carrageenan.

The paw volume upto the tibiotarsus was measured at 0, 1, 2, 3, 4, 5, 6. hr.

2.4. Statistical Analysis

The statistical analysis for the evaluation of the anti-inflammatory ointment against the Carrageenan induced paw edema in albino rats were analyzed using Anova and expressed as mean ± SEM. Difference between the mean of treated animals and control group were considered significant of P 0.05.

2.5. Carrageenan Induced Inflammation

Carrageenan induced inflammation is an acute test and is widely used as a model for the evaluation of anti-inflammatory activity of drug. Carrageenan induced edema is a biphasic event, with early hyperemia due to the release of histamine and serotonin and the delayed edema due to the release of bradykinin and prostaglandin [9].

The acute anti-inflammatory effect was evaluated by carrageen an induced hind paw edema. The edema was induced by injection of 1% suspension of carrageenan in 0.9% sterile saline solution into the rat right plantar region. Different group of animal was pretreated with the sample (1%AgNP ointment) 1hr before eliciting paw edema. Rat's paw volumes were measured by digital plethysmometer.

Measurement was done immediately at different time interval following Carrageenan injection. The edema inhibitory activity was calculated according to the following formula

$$\text{Edema (\%) inhibition} = (1 - D/C) \times 100$$

Where,

D– Represents the % difference in increased paw volume after the administration of test drugs to the rats.

C– Represent the % difference of increased volume in control group.

3. Wound Healing Activity of Functionalized Silver Nanopartices Ointment

Wound healing is a complex process with series of events that starts at the injury onset which continues for a long time mainly includes the stages hemostasis, inflammation, and repair and is the subject matter for in-depth-research [10]. Wound healing means faster recovery, minimal scar and better cosmetic appearance. Wound healing proceeds through numerous organized series of networks which include coagulation, inflammation at the sites, and proliferation of the aggregating factors and restructuring of tissues [11].

Therefore the percentage closure of original wound area is calculated in mm^2 at different time intervals. The various parameters monitored include:

a. Wound contraction studies.
b. Complete epithelialization study.
c. Collagen content.

3.1. Study Setting

The study was conducted in the Balpande college of Pharmacy, Nagpur, India. After obtaining necessary approval from Institutional Animal Ethics Committee (Ref. no DBCOP/30/31A/2017).

3.2. Material and Methods

AgNP ointment of 1% is used for the experiment of wound healing in animal model. Ketamine and xylazin injection was used to induce anesthesia. Other material required in the experimentations are polythene paper, graph paper, 4–zero silk thread, straight round needles, cotton, Allies forceps, straight scissors, scalpel applicators etc. The test samples; standard and control were applied to the wound models by means of applicator.

Group 1: Control treated with simple ointment base.

Group 2: Standard treated with silver sulfadiazine ointment.

Group 3: Test 1 treated with silver nanoparticles.

Application was done on animals on daily bases till complete healing.

3.3. Selection of Animals

Albino rats of 150-200g weight were used for experimental study. The study was approved by institutional animal ethical committee 1426/PO/Re/S/11/CPCSEA; Date: 1/08/2016. The animal was kept in the laboratory for 7 days for acclimatization with free access to food and water. Rats were housed in group of six in clean acrylic cages; these were cleaned every day. The animal was maintained under natural day and night cycle. These animals were segregated in three different groups. 1st group was treated with simple ointment base. 2nd group was "standard" treated with silver sulfadiazine (1%) cream. 3rd groups were treated with functionalized AgNP with 1% ointment formulations respectively. The application of AgNP was done once daily bases to the excision wound model animals for 10 days at a specified time and monitored till the wound is completely healed.

3.4. Experimental Procedure for Excision Wound Method

On the first day, animals were anaesthetized and brought to operation table. On the dorsal thoracic central region near ears, 5mm marking is done by using around seal of 2.5cm diameter. To get a wound area of $500mm^2$, the skin was cut throughout the thickness of skin. Wound was blotted with cotton swab soaked in normal saline solution to bring it to homeostasis condition. Then rats were placed back into individual cages. The wound closure process of healing was physically observed and termed as contraction which mainly contributes for wound closure, was studied. Raw wound area was traced on a polythene paper on wounding date followed by 4, 8, 12, 16 and 20th day after removing scar till complete epithelialization occurred; Area of wound was measured on tracing the lesion on a millimeter graph paper.

Following formula was used to calculate the rate of wound healing as % closure of the original wound area.

$$\text{Percentage closure} = 100 - [AD/AO * 100]$$

Where,

AO = 1st day wound area and

AD = corresponding day wound area.

The mean value so far wound areas were calculated. For complete epithelialization, required amount of days was recorded.

4. Estimation of Collagen Content in Granulation Tissue

Healthy male and female albino rat's 150-200g wt. is used for the experiment. The rats must be segregated into three groups each containing 3 animals. The wound induction and drug treatment is similar to the excision method.

4.1. Procedure

The regenerated tissues extracted from the open wound is collected from the animals and they were sacrificed at 4, 8, 12, 16 and 20th post wounding day.

4.2. Method of Collagen Estimation

The regenerated tissue collected from the excision wounds is cut into two pieces. They are washed with 0.5M sodium acetate and then suspended in 10 parts w/v of 0.5 acetic acid and stirred intermittently for 48hrs. The solution is centrifuged at 5600rpm for 2h (intermittently) in the micro-centrifuge, and

then sodium chloride 5% w/v solution is added to precipitate the collagen. The collagen so precipitated is filtered using a pre-weighed Whatman Filter Paper-No. 1. The weight of the collagen precipitate obtained is calculated by taking difference between the initial and the final weights of the filter paper. The same procedure was followed for the animals of the control and for the test groups.

4.3. Observation

Necropsy: A gross necropsy was performed on all animals that were sacrificed at the termination of the test.

Blood chemistry: Blood Samples were collected separately in tubes for clinical chemistry on the day of sacrifice of the animals. Samples were analyzed by Pathozyme Blood Chemistry analyzer.

5. Result for Anti–Inflammatory Activity of Ointments in Experimental Rats

Table 1. *(Control) Carrageenan.*

Animal	Initial Paw	1st hr	2nd hr	3rd hr	4th hr	5th hr	6th hr
1	0.45	1.15	1.51	1.69	1.83	1.72	1.52
2	0.68	1.38	1.71	1.9	2.07	1.95	1.65
3	0.97	1.57	1.87	2.05	2.19	2.08	1.88
4	0.99	1.49	1.8	1.97	2.12	2.01	1.81
5	1.03	1.53	3.33	3.61	3.76	3.64	3.44
6	1.11	1.41	1.68	1.8	1.9	1.1	0.9

Table 2. *(Standard) Standard Diclofenac.*

Animal	Initial Paw	1st hr	2nd hr	3rd hr	4th hr	5th hr	6th hr
1	0.33	1.42	0.88	0.79	0.66	0.57	0.52
2	0.36	1.44	0.89	0.81	0.72	0.64	0.58
3	0.57	1.63	1.07	0.98	0.87	0.80	0.75
4	0.61	1.65	1.1	1.03	0.96	0.89	0.85
5	0.79	1.83	1.27	1.19	1.07	0.99	0.93
6	1.19	2.24	1.67	1.58	1.45	1.36	1.31

Table 3. *(Test 1) Ointment containing AgNP 1%.*

Animal	Initial Paw	1st hr	2nd hr	3rd hr	4th hr	5th hr	6th hr
1	0.28	0.87	1.02	0.79	0.62	0.54	0.45
2	0.42	1.00	1.15	0.92	0.79	0.67	0.58
3	0.49	1.08	1.22	0.97	0.8	0.65	0.55
4	0.77	1.37	1.52	1.26	1.07	0.9	0.78
5	1.16	1.87	2.04	1.75	1.55	1.36	1.21
6	1.22	1.94	2.11	1.81	1.6	1.4	1.23

5.1. Result for Mean Increase in Paw Volume (% Inhibition of Paw Edema)

Table 4. *Mean and standard deviation of the entire groups.*

grp		Int. paw	1st hr	2nd hr	3rd hr	4th hr	5th hr	6th hr
Con	mean	0.87	1.42	1.98	2.17	2.31	2.28	1.86
	+ sd	0.25	0.15	0.67	0.71	0.72	0.84	0.84
Std	mean	0.64	1.70	1.14	1.06	0.95	0.87	0.82
	+ sd	0.31	0.30	0.29	0.29	0.28	0.28	0.28
Test 1	mean	0.72	1.35	1.51	1.25	1.07	0.92	0.80
	+ sd	0.39	0.45	0.46	0.43	0.41	0.37	0.34

5.2. Results for Percentage of Inhibition

Table 5. *Percentage of inhibition of all the groups.*

Group	Initial Paw volume	6hr	Difference in Paw	Inhibition percentage
Control	0.87	1.86	0.99	…..
Standard	0.64	0.82	0.18	81%
Test1	0.72	0.80	0.08	91%

Anti-inflammatory effect of silver nanopartices ointment against carrageenan induced paw edema is shown in Table 5.

The doses of Silver nanoparticles containing 200nm 1% showed statistically significant ($p<0.001$) inhibitory effect on "mean increase in paw volume" at all-time intervals (1, 2, 3 and 4 hours). Maximum percentage inhibition was observed at the end of four hours. The test doses 2000mg/kg body weight reduced the edema induced by carrageenan by 91%. The

standard drug showed 81% of inhibition compared to control.

6. Result for Wound Healing Study Using Ointment of Prepared Silver Nanoparticles

Result on 4th day showed that Ointment containing 1% Silver nanoparticle showed significant (P<0.01) wound closure 369.58mm^2 (26.71%) compared to control group 368.75mm^2 (25.71%), on 8th day showed significant (P<0.01) wound closure 159.58mm^2 (68.33%) compared to control group 261.25mm^2 (47.38%), 12th day showed significant (P<0.01) wound closure of 93.75mm^2 (81.38%) compared to control group 143.75mm^2 (71.17%), on 16th day showed significant (P<0.01) wound closure around 60.41mm^2 (88.01%) compared to control group 83.33mm^2 (83.22%) and on 20th day Ointment containing 1% Silver nanoparticle showed significant (P<0.01) wound closure 60.41mm^2 (95.28%) compared to control group 45.83mm^2 (90.36%).

Table 6. *Photos showing excision wound.*

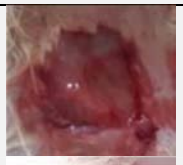
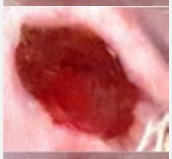
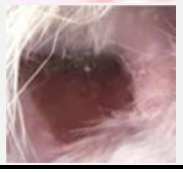
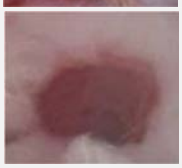
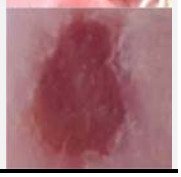
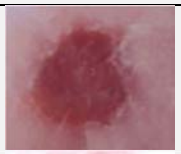
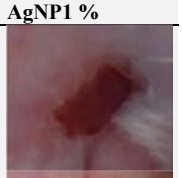
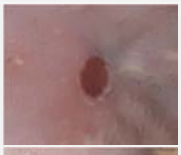
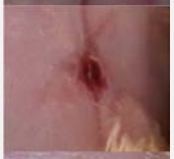
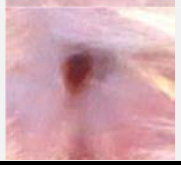
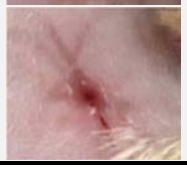
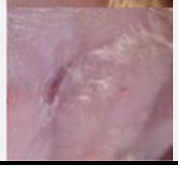
	Control	Std	AgNP1 %
0Day			
4Day			
8Day			
12Day			
16Day			
20Day			

Table 7. *Mean wound area (RWA) mm^2 of closure of excision wound at different time interval.*

Day	Mean Raw Wound Area (mm^2)		
	Control	Standard	Test (AgNP1%)
0	495.83	497.9	504.16
4	368.75	360.41	369.58
8	261.25	185.41	159.58
12	143.75	129.16	93.75
16	83.33	68.75	60.41
20	45.83	31.25	22.16

Table 8. *Mean % of closure of excision wound at different time interval.*

Day	Mean Percentage (%) closure		
	Control	Standard	Test (AgNP1%)
0	……	…..	…..
4	25.71	27.61	26.71
8	47.38	62.75	68.33
12	71.17	74.05	81.38
16	83.22	86.18	88.01
20	90.36	93.71	95.28

Statistical analysis of the result obtained at 20th post wounding day by ANOVA test showed that there was significant difference between the entire group, P<0.05 and the silver nanoparticle of 1% was found to be highly effective concentration.

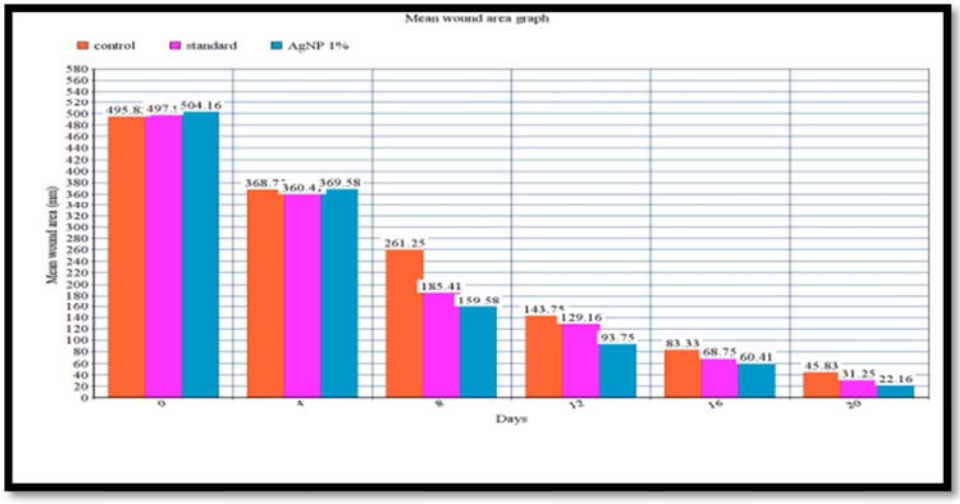


Figure 1. *Showing mean raw wound area of excision wound area at different time interval in various groups.*

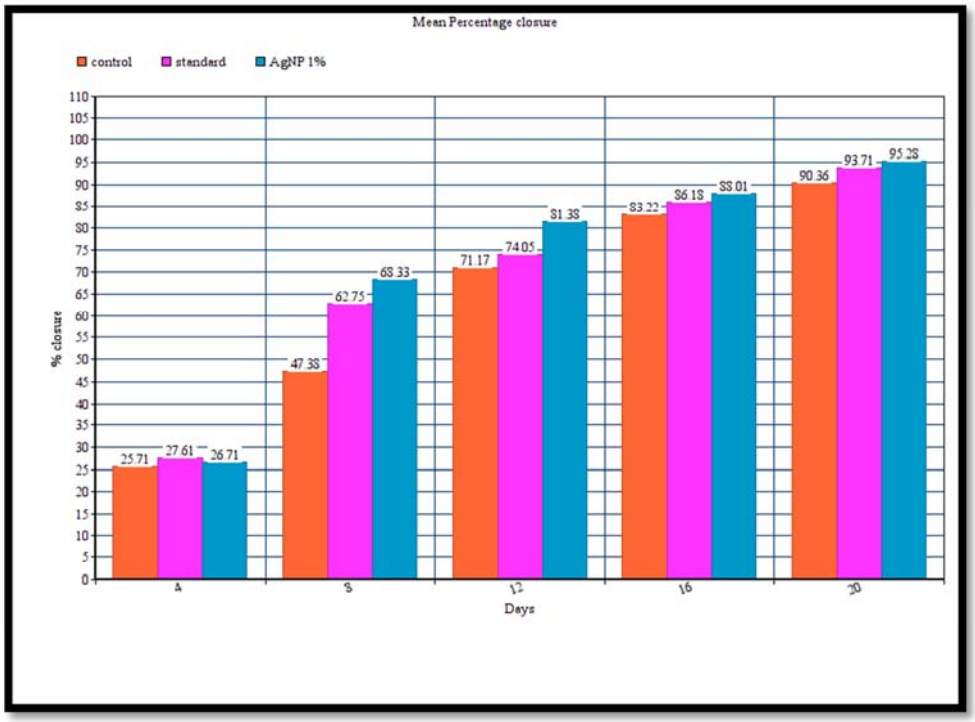


Figure 2. *Showing % of Closure of excision of wound area at different time intervals in various groups.*

6.1. Result for Complete Epithelialization

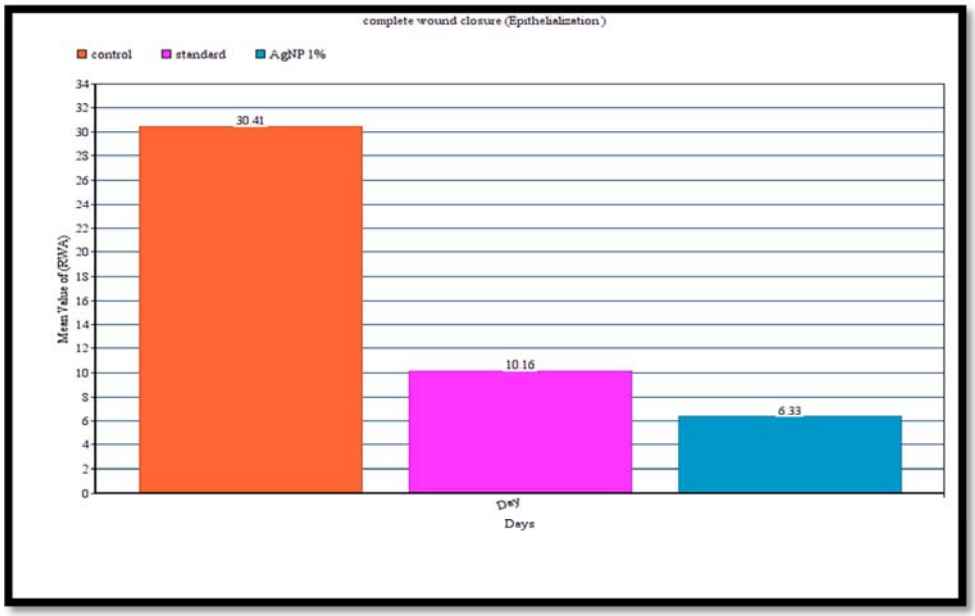


Figure 3. *Time (Days) for complete wound closure (Epithelialization) of excision wound.*

Table 9. *Time (Days) for complete wound closure.*

Animal no.	Raw wound area		
	Control	standard	Test1 (AgNP1%)
1	20	12	10
2	39	9	6
3	19	6	3
4	18.5	15	9
5	38	5	3
6	48	14	7

Animal no.	Raw wound area		
	Control	standard	Test1 (AgNP1%)
Mean	30.41	10.16	6.33
+SD	12.81	4.16	2.94

(Epithelialization) of excision wound.

On further follow-up, mean time (days) to complete healing of control was (30.41) while that of AgNP 1% treated group was (6.33), and that of standard was (10.16) indicating

significant epithelialization compared to control.

6.2. Results for Collagen Content

Table 10. *Mean Collagen content from regeneration Tissue of excision wound (mg/kg).*

Days		control	Std.	AgNP 1%
4	Mean	9.35	21.55	23.8
	+SD	0.85	0.61	0.89
8	Mean	14.7	31.06	33.61
	+SD	0.86	1.25	0.75
12	Mean	20.83	38.26	41.1
	+SD	1.63	0.91	0.98
16	Mean	29.35	43.05	47.41
	+SD	1.10	0.44	0.81
20	Mean	37.7	51.43	55.45
	+SD	0.92	0.80	0.75

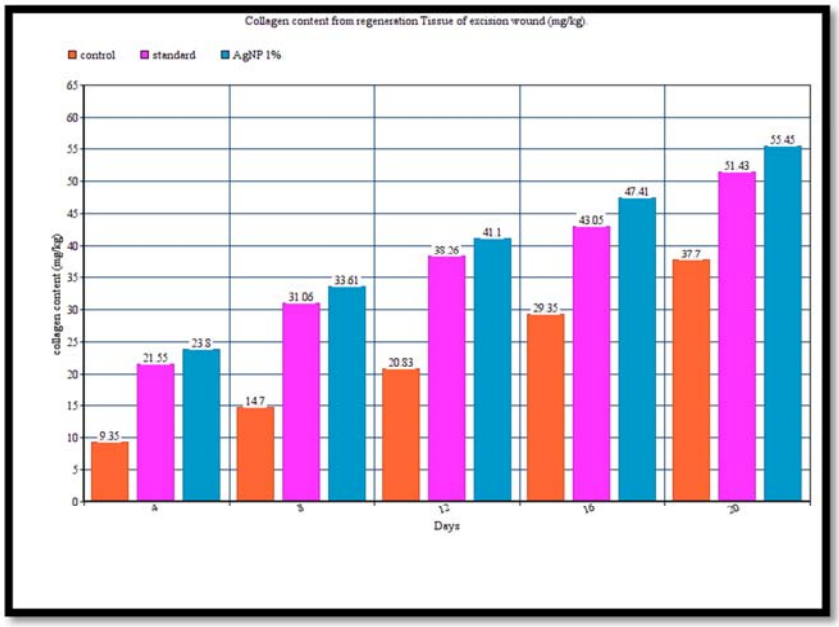


Figure 4. *Collagen content from regenerated Tissue of excision wound (mg/Kg).*

6.2.1. Result for Mean Hematology Data

Collagen content was analyzed from the regenerated tissues for control as well as treated group. There was a significant increase in collagen content during study in 1% AgNP ointment group compared to control and standard.

Statistical analysis of the result by ANOVA showed that there was significant difference between the entire group and the 1% AgNP ointment was found to be highly effective.

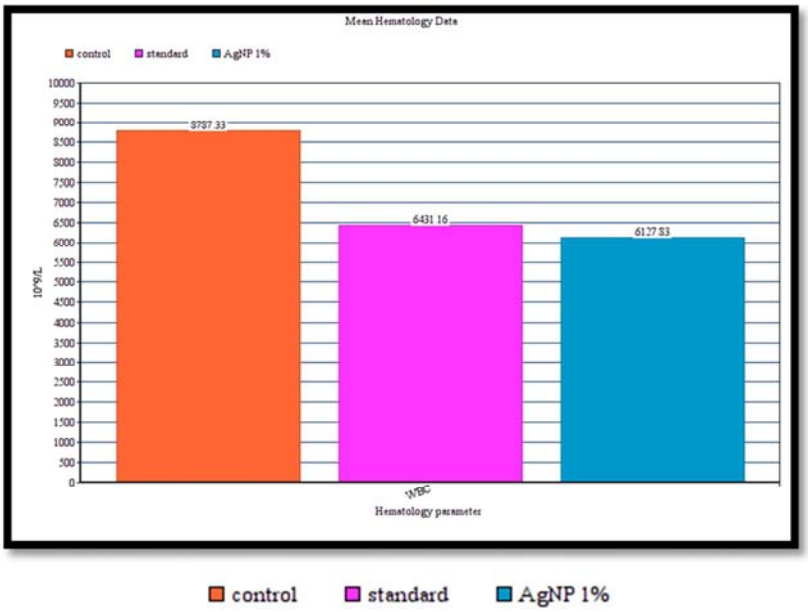


Figure 5. *WBC.*

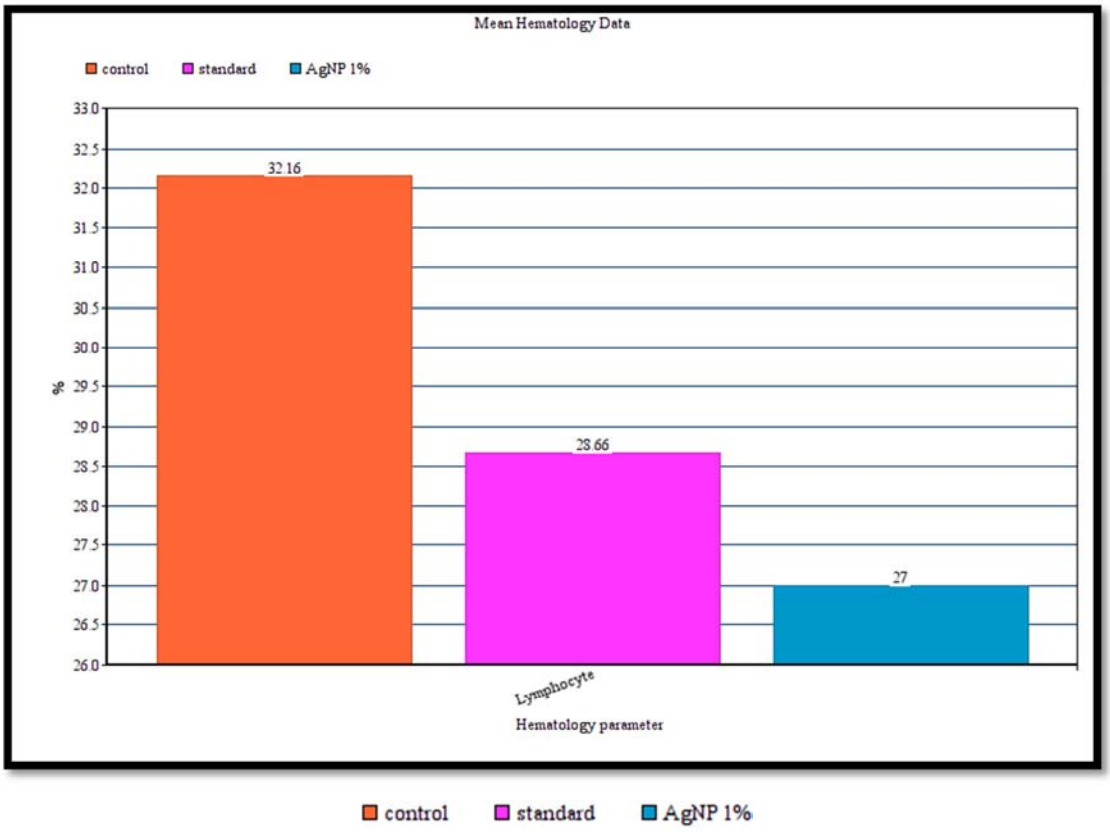


Figure 6. *Lymphocyte.*

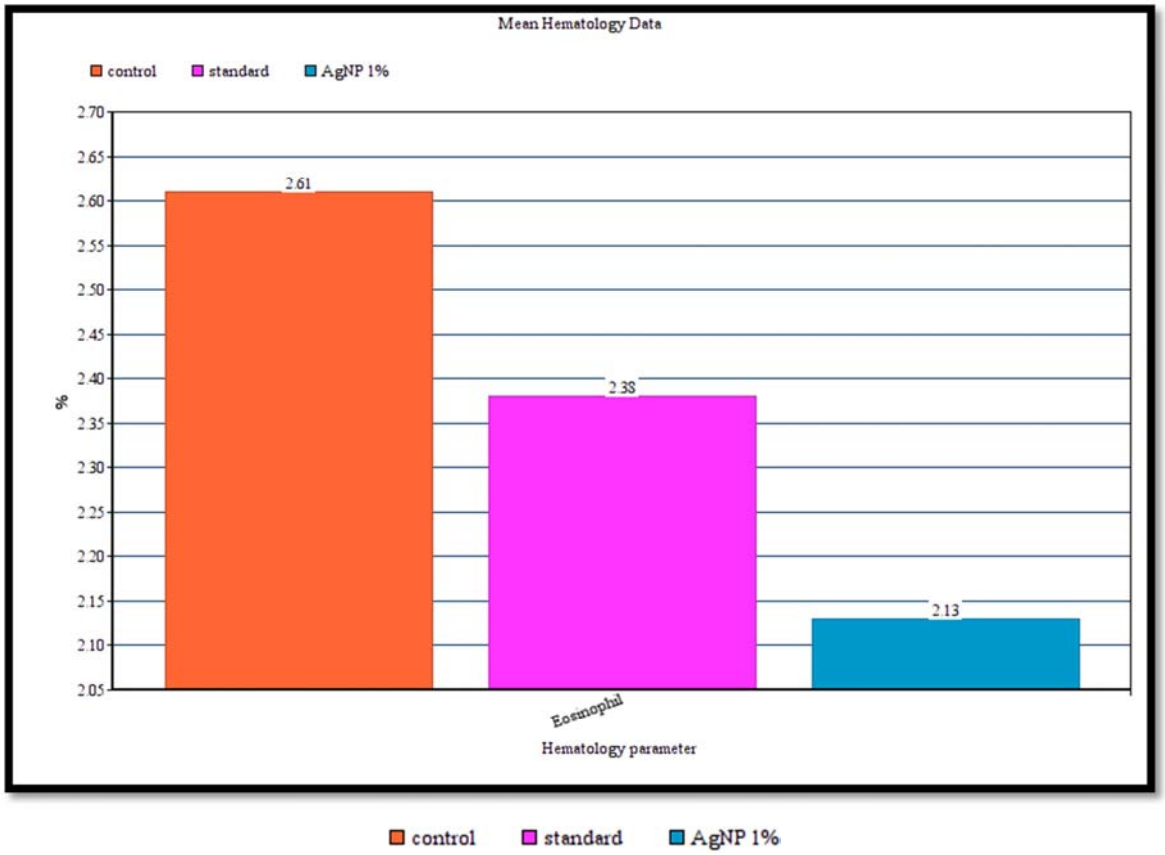


Figure 7. *Eosinophil.*

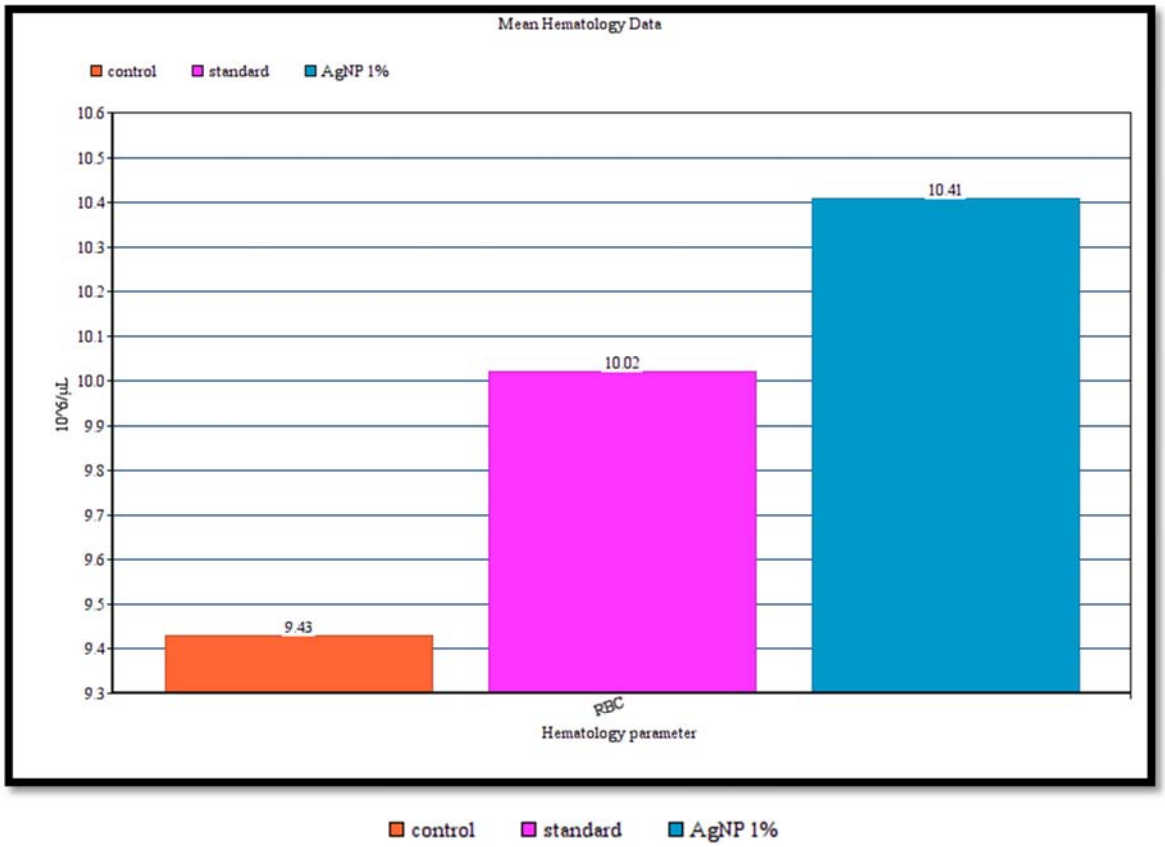


Figure 8. *RBC.*

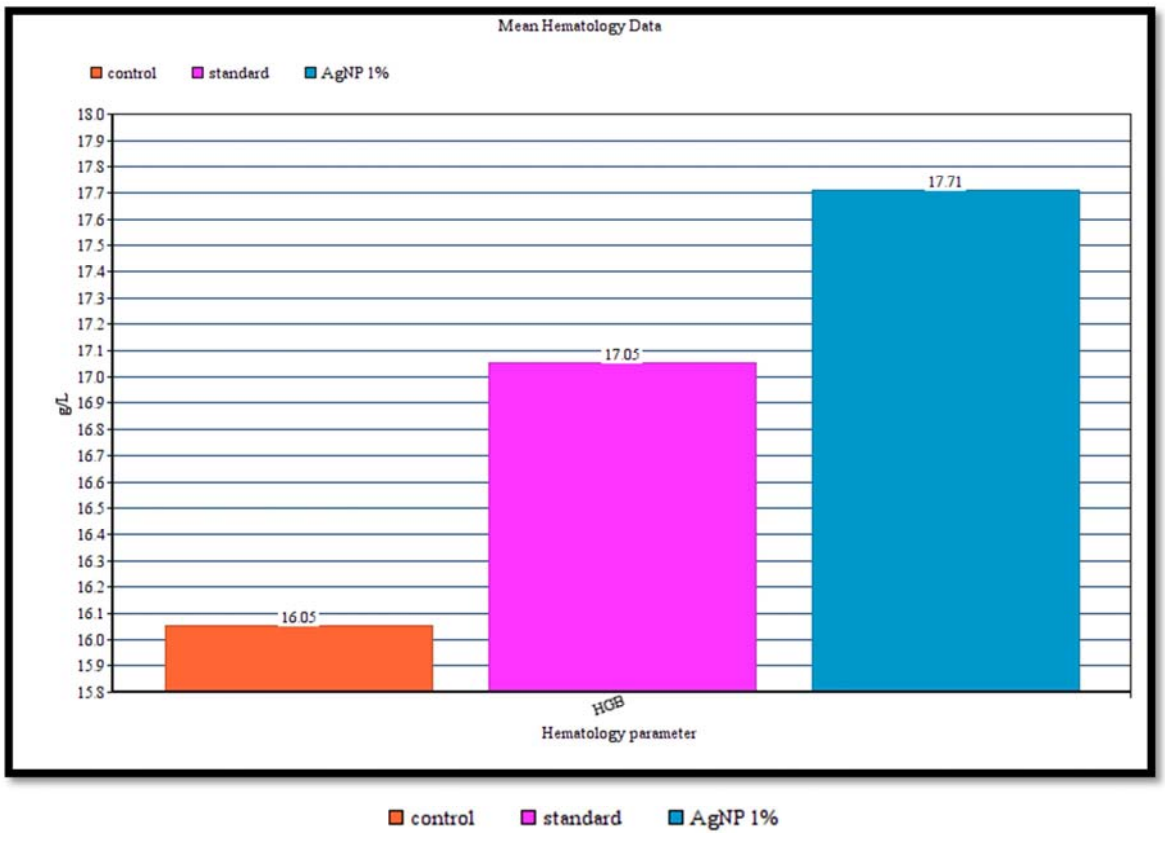


Figure 9. *HGB.*

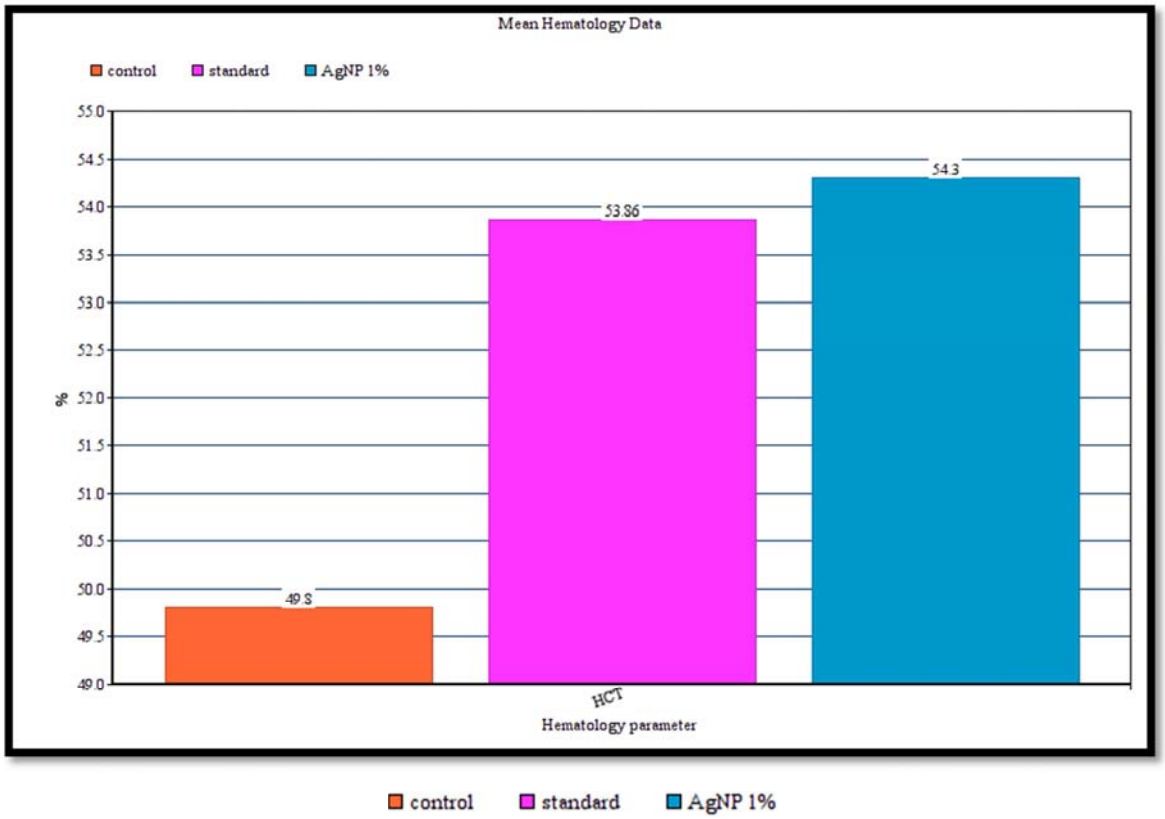


Figure 10. *HCT.*

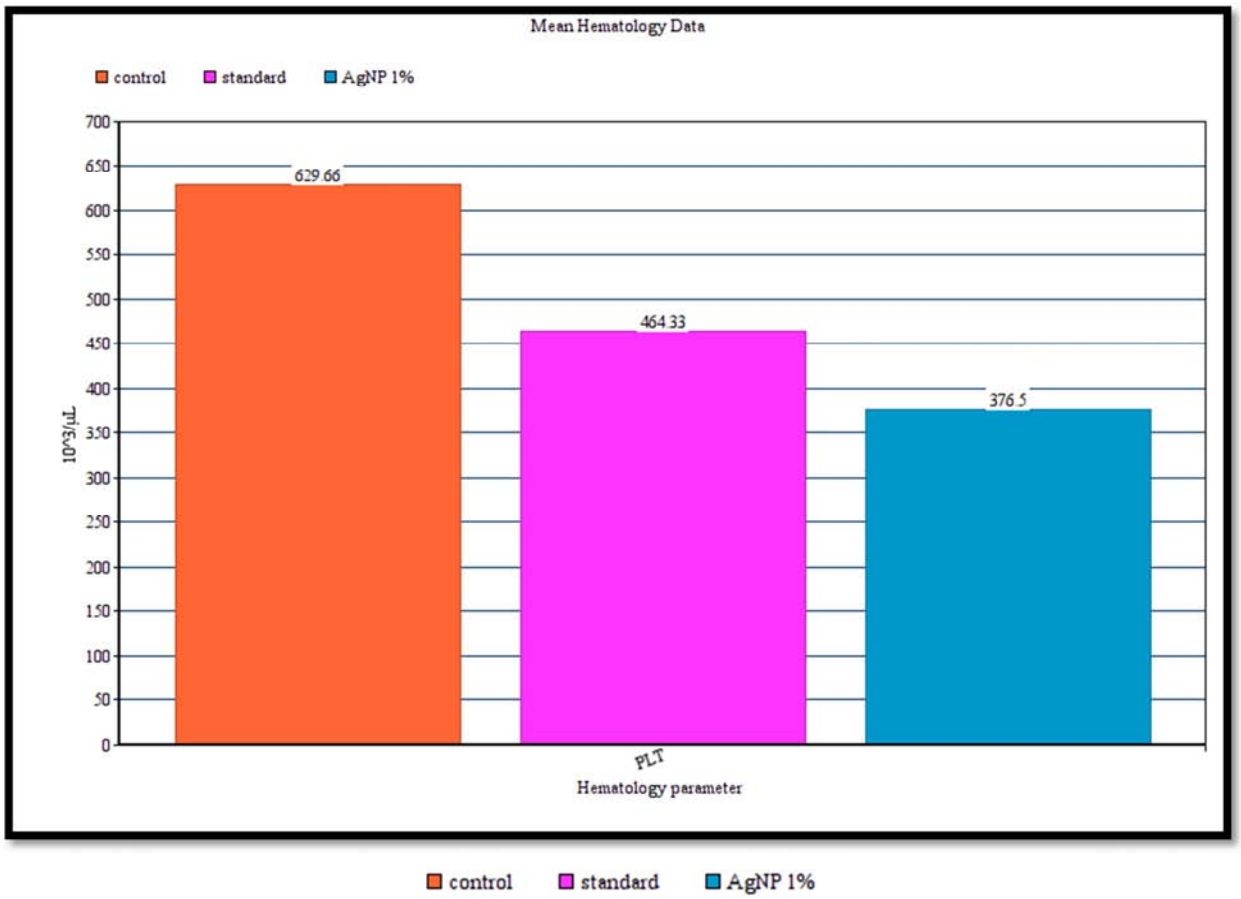


Figure 11. *PLT.*

Table 11. *Group Mean Hematology Data.*

Group		WBC	Lymphocyte	Eosinophil	RBC	HGB	HCT	PLT
Control	Mean	8787.33	32.16	2.61	9.43	16.05	49.8	629.66
	+SD	601.11	1.72	0.07	0.72	1.23	3.51	114.31
Standard	Mean	6431.16	28.66	2.38	10.02	17.05	53.86	464.33
	+SD	530.13	1.63	0.11	0.63	1.54	4.37	185.52
AgNP1%	Mean	6127.83	27	2.13	10.41	17.71	54.3	376.5
	+SD	400.62	1.78	0.12	0.34	0.80	1.88	90.94

6.2.2 Result for Mean Biochemistry Data

Table 12. *Group Mean Biochemistry Data.*

Group		TP gm/dL	ALB gm/dL	ALP	SGOTIU/L	SGPTIU/L	UREA mg/dL	CHOL Mg/dL	TGL Mg/dL
Control	Mean	6.86	4	27.66	171.5	79.66	32.7	0.85	61.33
	+SD	0.61	0.23	3.32	44.46	8.23	2.19	0.05	3.77
Standard	Mean	8.41	4.11	50.33	165.66	98	29.55	0.86	67
	+SD	0.32	0.20	7.60	27.2	14.18	4.52	0.121	9.48
AgNP1%	Mean	7.35	3.85	51.16	158.16	83.83	25.28	0.83	66.5
	+SD	0.90	0.45	5.91	37.62	13.49	4.38	0.10	6.34

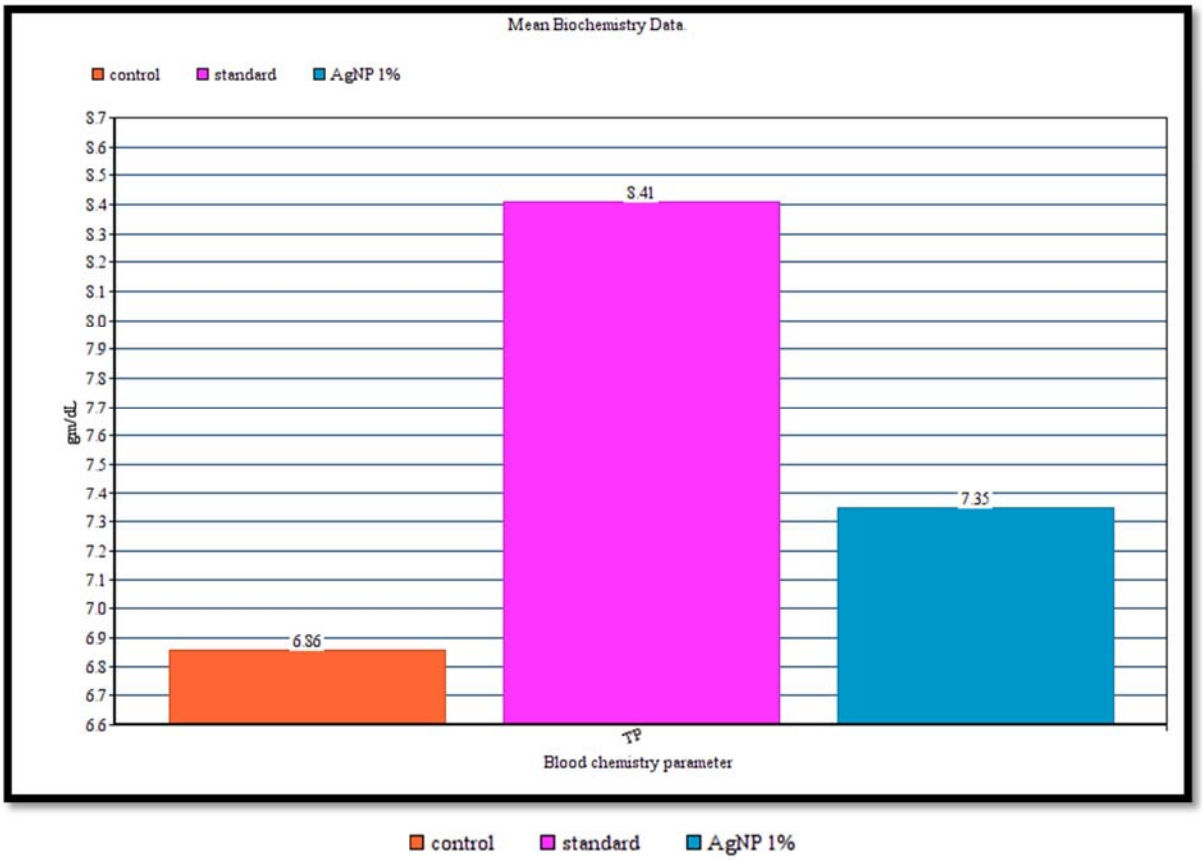


Figure 12. *TP.*

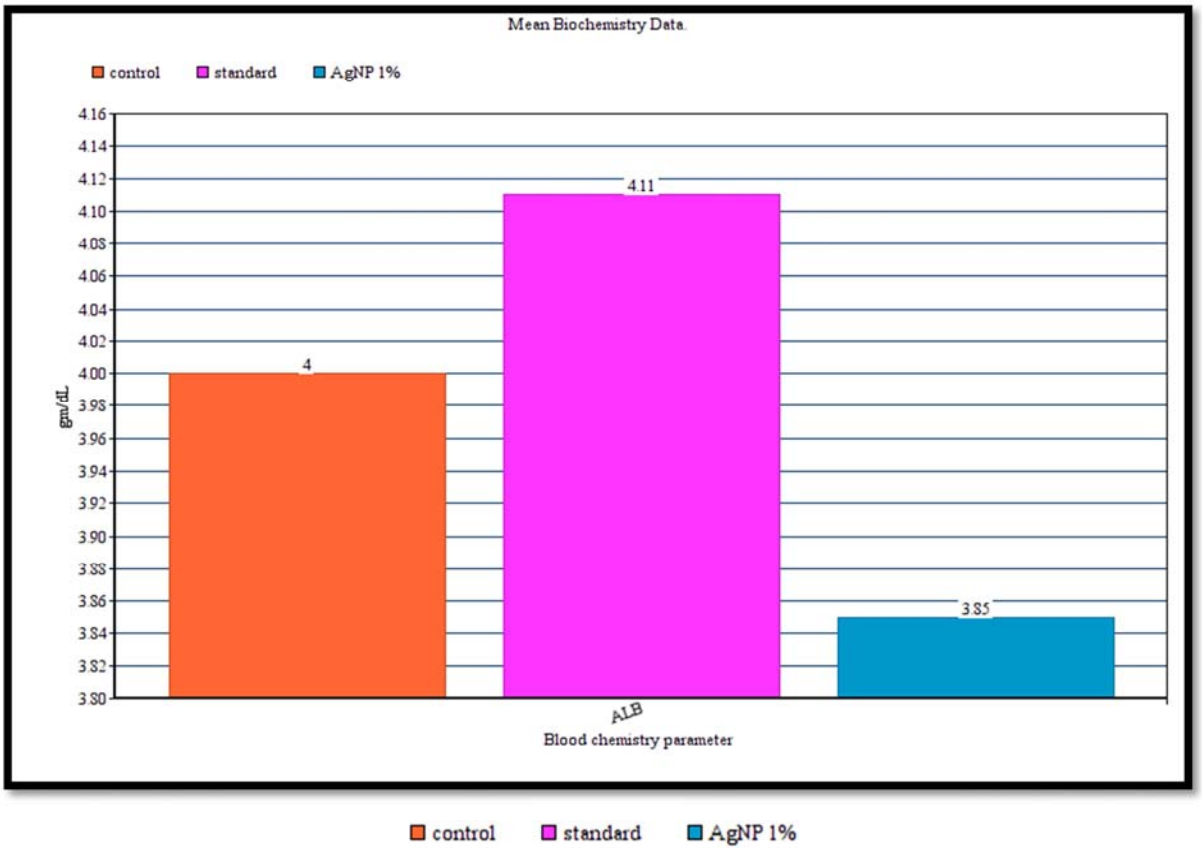


Figure 13. *ALB.*

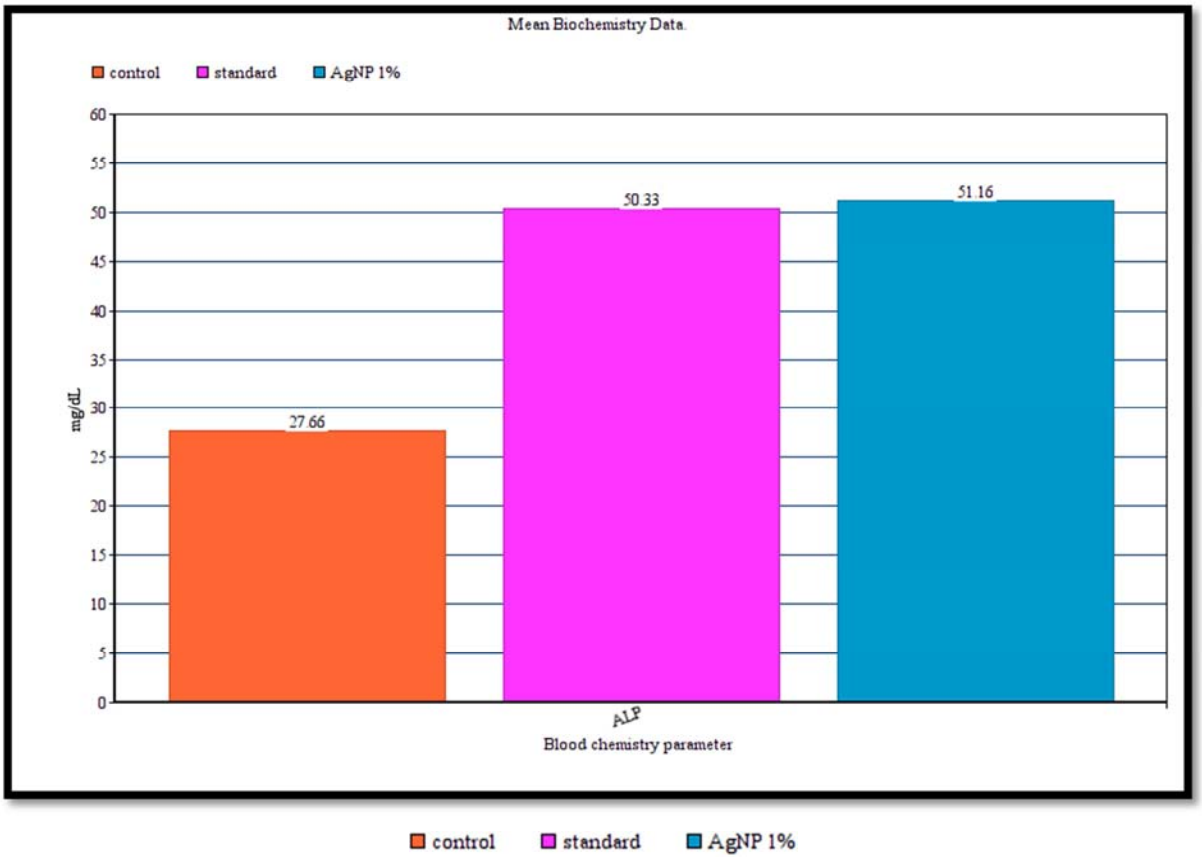


Figure 14. *ALP.*

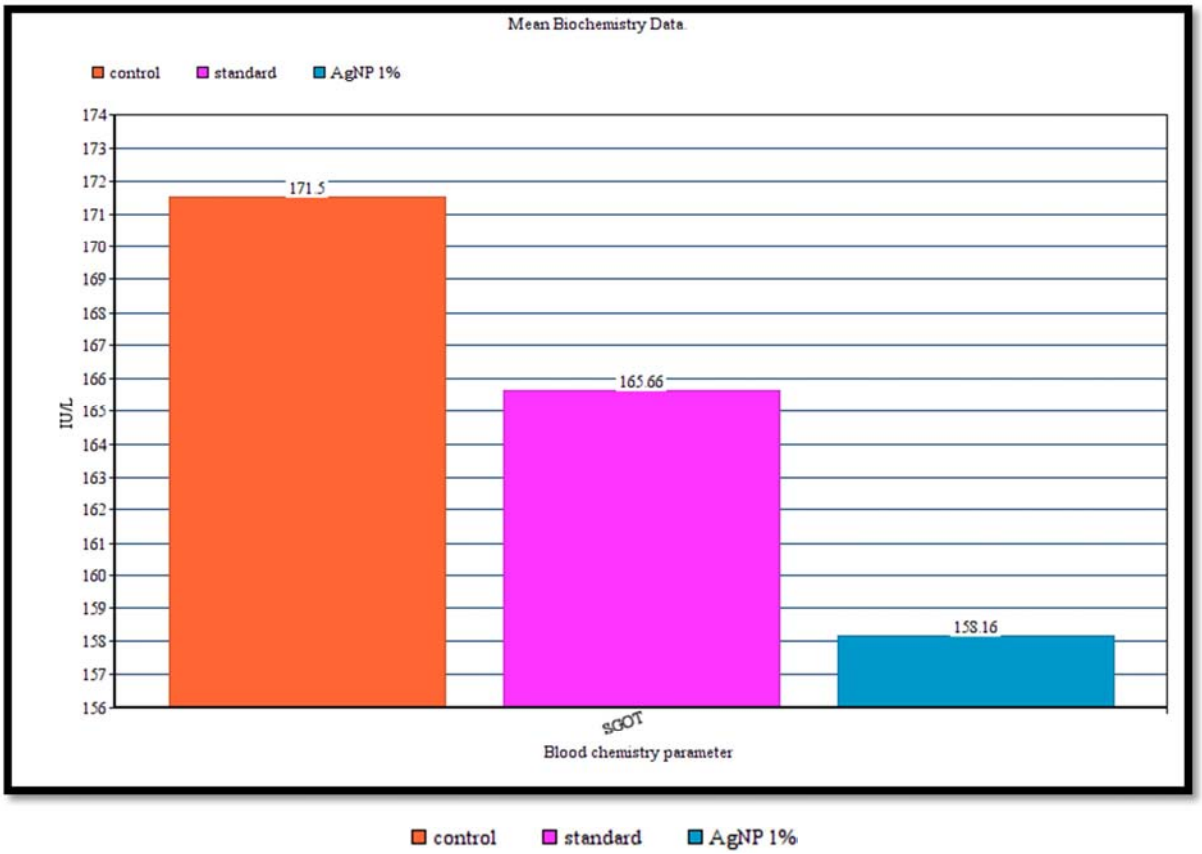


Figure 15. *SGOT.*

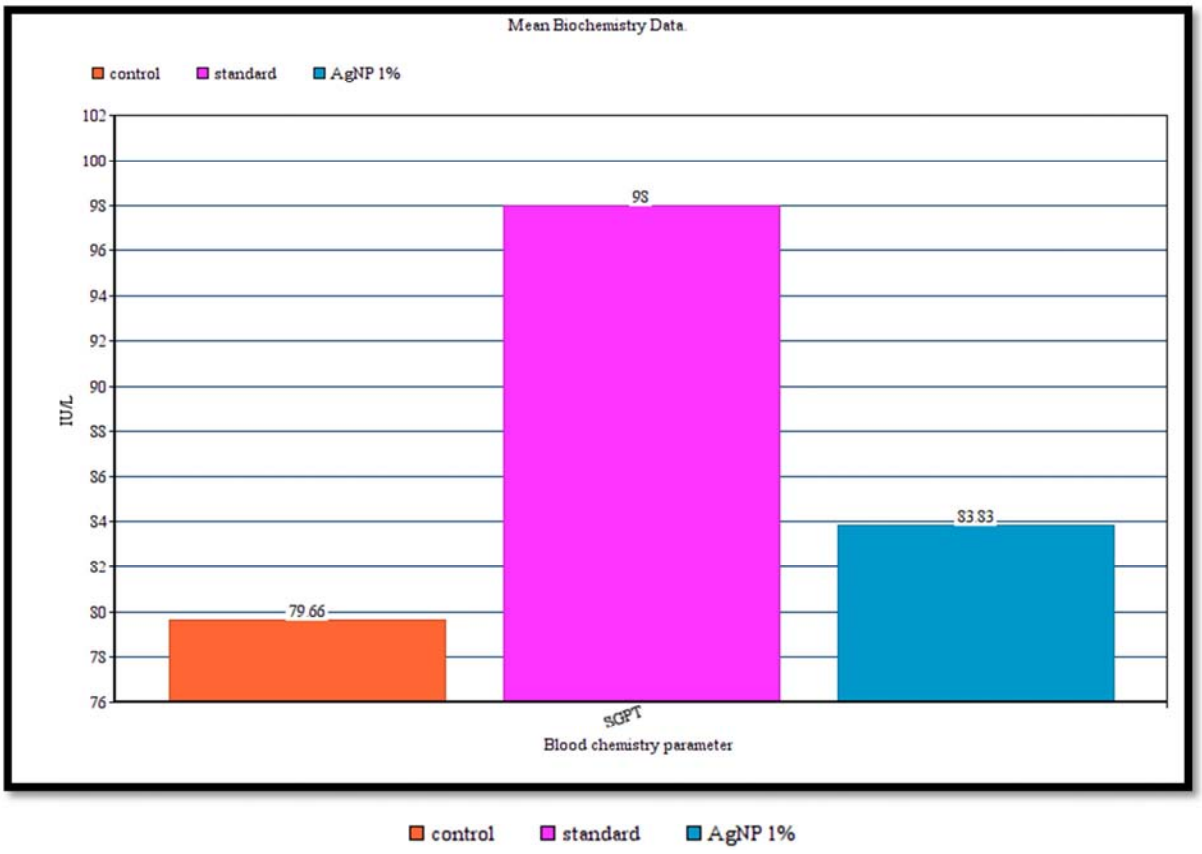


Figure 16. *SGPT.*

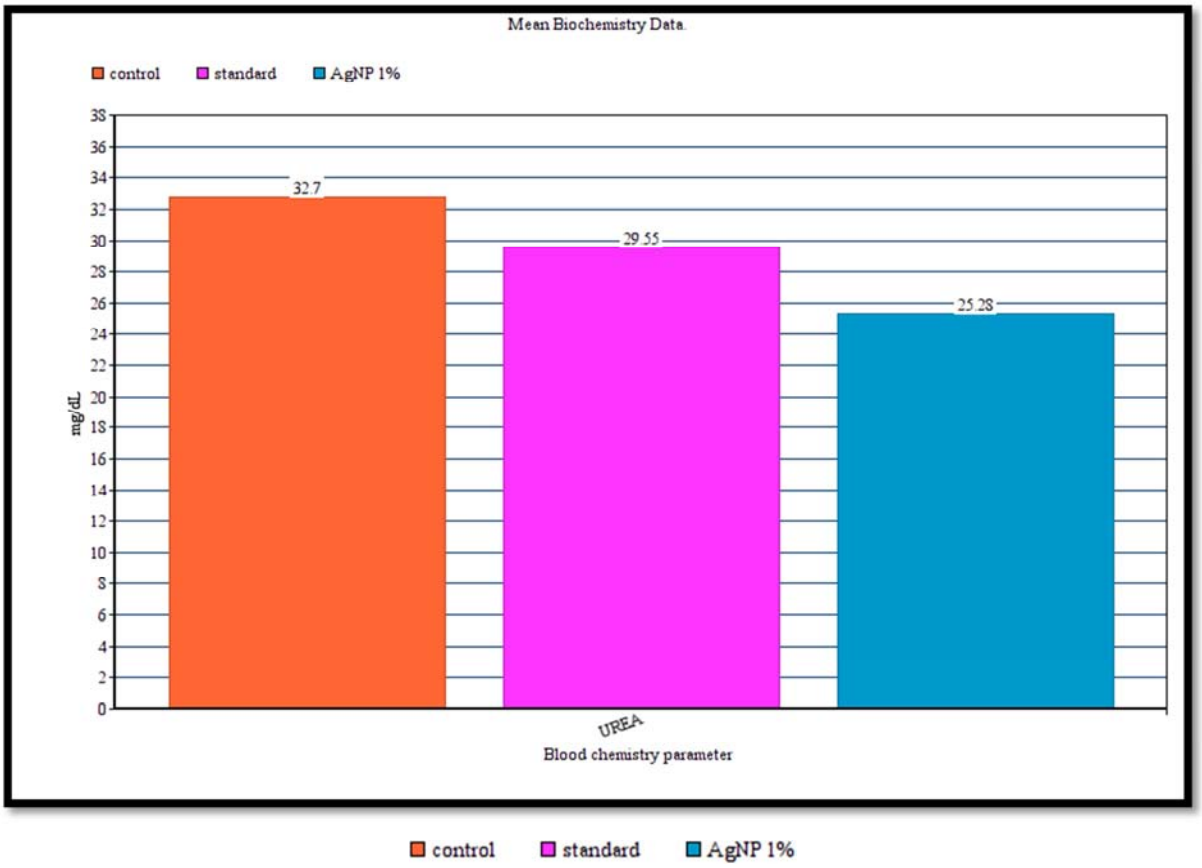


Figure 17. *UREA.*

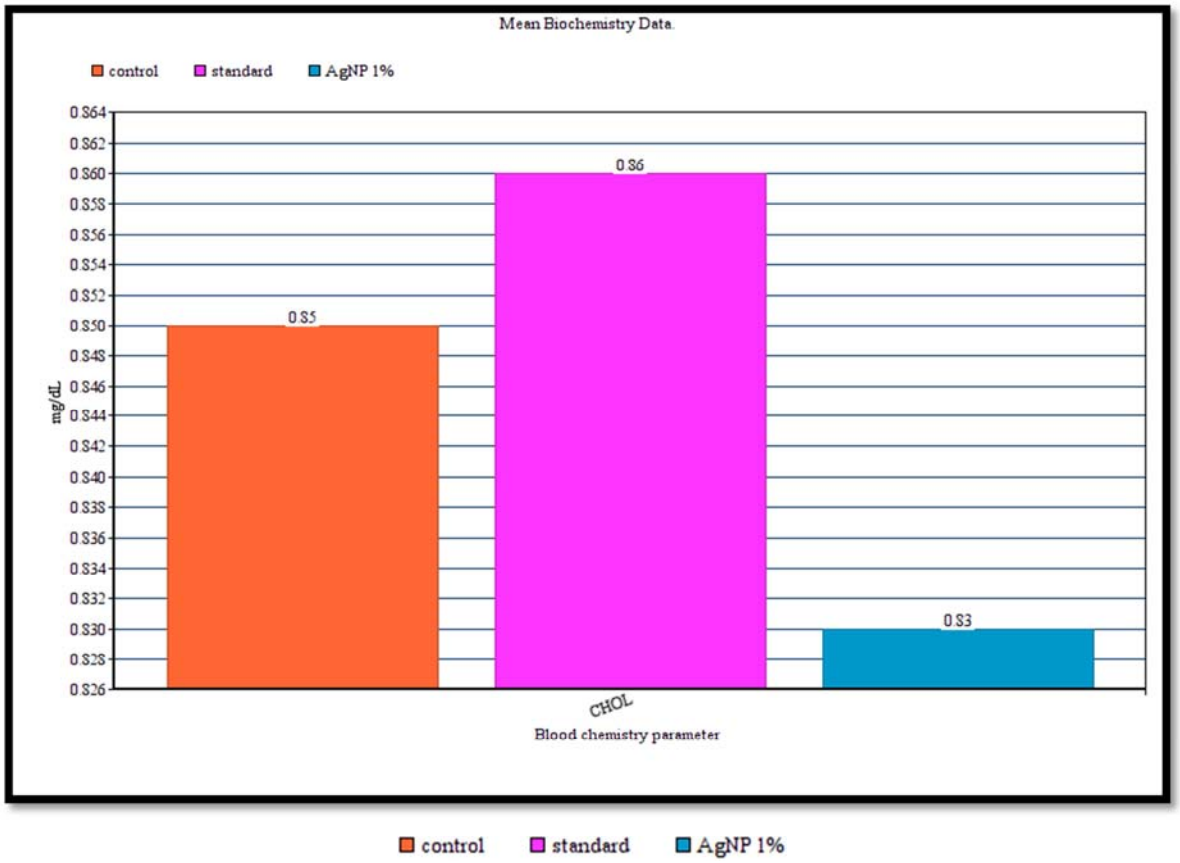


Figure 18. *CHOL.*

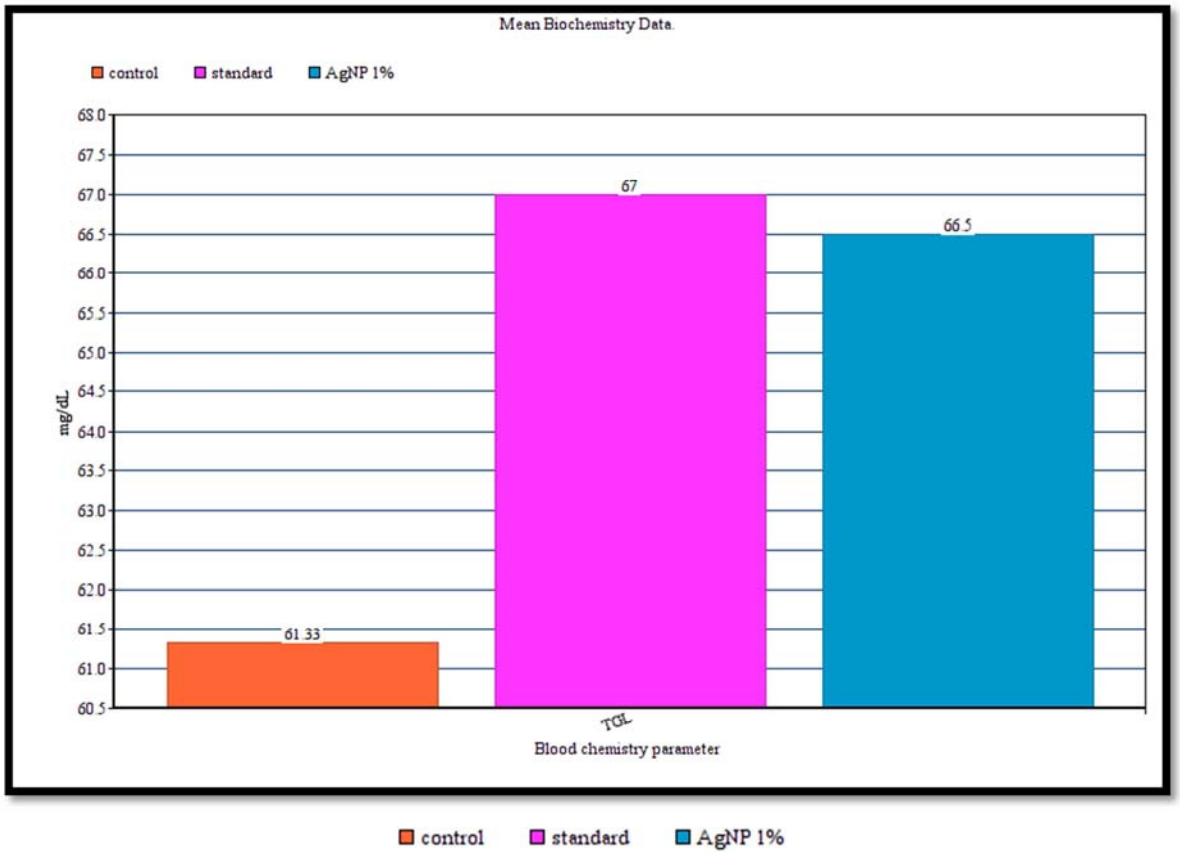


Figure 19. *TGL.*

7. Discussion

Carrageenan induced paw edema was characterized by biphasic event with involvement of different inflammatory mediators. First phase involves the release of histamine, serotonin, and kinins after carrageenan injection in first few hours. While in second phase prostaglandins are release [12]. As prostaglandins is responsible for acute inflammation and silver nanoparticles might containing anti inflammatory agent which might be responsible for blockage of prostaglandins and inflammatory pathway. Thus the results revealed that administration of 1% Silver nanoparticle ointment inhibit edema starting from the first hour and all phases of inflammation, which is probably due to inhibition of release of different chemical mediators of inflammation. Inflammation is considered as a fundamental process in early stage of acne development. It is also said that the anti-inflammatory and antibacterial both this agent helps in enhancing the efficacy against comedonal acne [13], as this both anti-inflammatory and antibacterial properties are present in silver nanoparticles, it can be used in acne preparation.

In further study ointment contains a silver nanoparticle which was tested for its wound healing properties. The wound area (RWA) (mm^2) and % of Closure of excision wound on 20^{th} wounding day showed significant result by ANOVA test, ($P<.01$) with P value being 0.00047 for wound area and 0.00042 for % closure. The mean wound closure of control was 45.83 and that of AgNP 1% showed better result with 22.16 whereas that of standard was found to be 31.25. This indicates that AgNP 1% ointment showed better result than the standard. The % closure of wound shows similar result to the wound area being 90.36% for control, 93.71% for standard and 95.28% for AgNp1%. On further follow-up, mean time (days) to complete healing of control was (30.41) while that of AgNP 1% treated group was (6.33), and that of standard was (10.16) indicating significant epithelialization compared to control. Normal wound healing is due to the orderly over lapping processes which repairs the skin function any alternation that impairs the normal healing process would result into tissue damage prolong repair process and result into the chornic wound healing, which can also be caused due to infection, diseases, medication and old age [14]. With this study we can state that silver nanoparticles when used in cosmetic preparation it can heal the minor wounds to chornic wound reduction because of the effective healing properties of silver nanoparticles which will further used to reduce the imperfection on the skin.

Collagen content was analyzed from the regenerated tissues for control as well as treated group. There was significant difference between the entire group and the 1% AgNP ointment was found to be highly effective. As the collagen synthesis is the first step due to the formation of precursor polypepetide such as lysine and proline residues. The remolding of scar tissue occurs due to the faster inter and intra molecular cross linking of collagen [15]. An elevated

level of hydroxyproline in the regenerated tissue is mostly responsible for enhanced collagen synthesis [16]. Collagen not only confirms strength and integrity to the tissue matrix but also plays an important role in hemostasis and epithelialization at a later phase of wound healing [17]. In control group the healing was slower due to the natural cell matrix interaction.

In aging skin the cell regeneration capacity reduces and the connective tissue (collagen, elastin, extracellular matrix) gets decline. This aging phenomenon reduces the healing mechanism of the skin [18]. Mostly the antiaging topical application are used to delay the aging signs and in our study we used the ointment containing silver nanoparticles, for studying relevant parameters such as wound healing especially emphasizing on collagen contain so that it can be relate to the collagen synthesis in the aging process. With this study we can state that the use of Silver nanoparticles in cosmetic formulation can help in reducing wrinkles and increase the firmness, soften skin as it can enhanced collagen synthesis. We can say that silver not only heal the wound but it also increase the collagen production which is why we can say it can be used in antiaging products in cosmetic as well. As in the wound healing study the collagen content can be seen in the final phase of wound healing is remolding phase which is surrounded by fibroblasts tissue which can be seen in 3rd week of injury. During the proliferation phase granulation tissue is formed which is immature type III collagen and during remolding this collagen is replaced mature type I collagen and gradually wound becomes less vascularized [19]. This helps skin look flawless. This was confirmed by the collagen and epithelization studies.

Overall the silver nanoparticle had significant therapeutic activity in excision wound study this might be due to the antimicrobial activity of silver nanoparticle. Acne treatment mostly used to reduce the acne lesions as well as improving the overall appearance. It also aims to reduce P. acnes proliferation and decreasing inflammation [20]. Since Silver nanoparticles have antimicrobial and anti-inflammatory properties it can be used in acne treatment.

In further study hematology analysis was done to examine the normal function of blood. There was slight change in the hematology parameter whereas, 1% AgNP showed better result in comparison to control and standard

Further Biochemistry data was analyzed to know whether or not the nanoparticle interacts with blood and affects the normal function. Result showed 1% AgNP ointment showed better result.

Overall the 1% AgNP ointment showed the better result in comparison to control and standard According to the result it can be stated that silver nanoparticle gives better result with normal function of the body and with no side effect.

8. Conclusion

Silver nanoparticles were synthesized by chemical reduction method with reduction and stabilizing agent. A specific control over size and shaped of silver nanoparticles was achieved. Overall silver nanoparticles had shown improved wound healing properties. Nano Silver has showed less adverse changes and accelerated re-epitheliazation and resulted in rapid complete healing of the wound.

Further it showed anti-inflammatory properties and universally accepted antimicrobial properties which can help in reducing acne to give flawless looking skin. It can also be used in antiaging products in cosmetic which can improve the skin health, as skin aging can be result of continuous imperceptible inflammatory assaults. Nano Silver can be used as in a healing cream as well, which make the silver as an active component that can be used as all purpose creams ingle component which can deal with the minor imperfection of the skin and also helps the skin look radiant.

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