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Lot B-G-34, Pangsapuri Sri Penara, Jalan Sri Permaisuri 1,
Bandar Sri Permaisuri, Cheras 56 000, Kuala Lumpur, Malaysia.
Tel/Fax: 03 9173 7022 Tel: 03-9172 6629

TEST REPORT

STUDY REPORT NUMBER: MB-**WH**-281h-07

Study Completion Date: 20th June 2009

Title: EFFICACY ASSESSMENT OF 'NATURAL SAGO HYDROGEL' FOR WOUND HEALING OF NORMAL RATS

Study Sponsor

Rumbia Bio-Tech Sdn Bhd
Blok 43 ALURTON,
MOSTI Agensi Nuklear Malaysia,
Jalan Dengkil, Bangi,
43000 Kajang,
SELANGOR.

Testing Facility

Animal Experimental Unit
Unit Makmal Haiwan,
Fakulti Perubatan Universiti Malaya,
60603 Lembah Pantai,
KUALA LUMPUR.

Study Director (1) : Dr. Saadiah Sulaiman

Study Director (2) : Assoc. Prof Dr. Md Mahmood Ameen Abdulla

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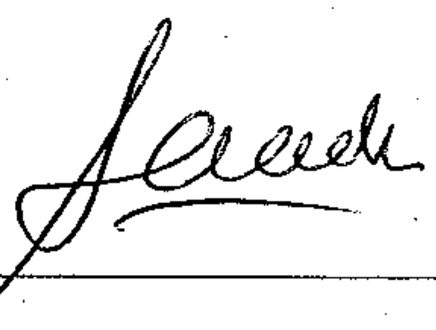
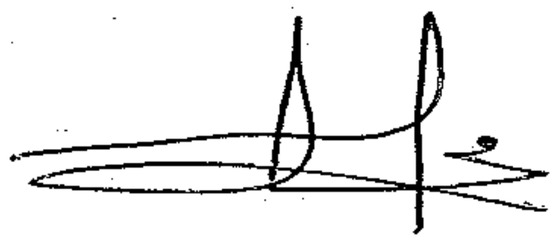
EFFICACY ASSESSMENT OF 'NATURAL SAGO HYDROGEL' FOR WOUND HEALING OF NORMAL RATS

Study Director (1)	**Signatures**
Dr Saadiah Sulaiman *MBBCh (Dub), MMED (UKM)*	 **Date:** 20/6/09
Study Director (2)	
Assoc. Prof. Dr. Md. Mahmood Ameen Abdulla *BVM&S (Mosul), M.Sc (Mosul), PhD (UPM)* Dr. Mahmood Ameen Abdulla Associate Professor Dept. of Molecular Medicine Faculty of Medicine, University of Malaya 50603 Kuala Lumpur	 **Date:** 20/6/09
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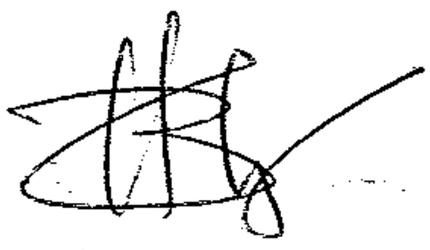
Rumbia Bio-Tech Sdn Bhd	 **Date:** 8/7/09

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TEST REPORT - Efficacy Assessment of NSH for Wound Healing of Normal Rats

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SUMMARY

Efficacy Assessment of NSH for Wound Healing of Normal Rats

Protocol reference : Protocol AL - WH

Study completion date : 26/6/09

Study reference number : WH-01-02-07

Study report number : MB-WH-281h-07

Job number : 281h-07-RBT

Test material : NSH

Conditions of use : Neat

1. **OBJECTIVE**
   To evaluate the efficacy of a test material named as Natural Sago Hydrogel following topical administration on wound of normal rats.

2. **EXPERIMENTAL PROCEDURE**
   Animals: Fifteen Sprague Dawley albino rats, (5 for negative control, female, 5 for positive control, all male).

Weight:	Test group	: 149 - 159 g
	Negative control group	: 156 - 168 g
	Positive control group	: 182 - 205 g

Date of initiation : 15th August 2007
Date of completion : 1st March 2008

Treatment
A full-thickness circular skin graft with a surface area of 5.66 cm^2 was harvested from the dorsum of each rat using surgical scissors. The full-thickness skin defect was left to heal by secondary intention, and each rat was kept in a separate cage with the defect uncovered.

The rats were divided into 3 groups of 5 rats. The first group was the study group and was treated with NSH topically. The second group was the negative control and left untreated and the third group was the positive control and was treated with Intrasite gel as positive control. All topical application was done once daily until the wound completely healed.

3. **OBSERVATIONS**

Clinical Examination

Animals were observed for improvement of wound daily. The day the wound is completely healed is taken as the end point.

Histopathological Examination

Upon healing, a full thickness biopsy of the healed wound is excised for histopathological examination.

4. **RESULTS**

The group treated with NSH took a significantly shorter duration of time for complete wound healing, i.e 12.4 days compared to 15.8 days for negative control, $p=0.000$ (i.e $p<0.05$). This group also had a shorter duration for complete wound healing compared to positive control that took 12.8 days but the difference was not significant, $p=1.000$ (i.e $p>0.05$). Positive control took a shorter duration for complete wound healing compared to negative control, $p=0.000$ (i.e $p<0.05$).

There was histopathological changes observed in all groups i.e group treated with NSH, untreated group (negative control) and group treated with Intrasite gel (positive control). NSH promotes wound healing activity in this excision wound model study, which is more or less equal to positive control (standard treatment- Intrasite gel). In all NSH treated wounds, the histological appearance of skin showed absence of inflammatory cells, increased collagen formation, fibroblast cells, new blood capillaries (angiogenesis) and re-epithelialization compared to the negative control. Although there were differences between NSH and Intrasite gel treated groups, but this difference was not significant. Granulation tissue of treated animals showed moderate deposition of collagen comparable to Intrasite gel treated animals. Scar area in wound closure was also noted to be smaller with NSH and Intrasite gel compared to negative control.

5. **CONCLUSION**

NSH is effective in promoting wound healing in normal rats, significantly shortens the duration of wound healing, and significantly promotes wound healing activity in the excised wound model. It is as effective as Intrasite gel in promoting wound healing in normal rat.

TEST REPORT

1.0 SPONSOR OF TEST MATERIAL

1.1	**Name**	: Rumbia Bio-Tech Sdn Bhd.
1.2	**Address**	: Blok 43 ALURTON, MOSTI Agensi Nuklear Malaysia, Jalan Dengkil, Bangi, 43000 Kajang, SELANGOR
1.3	**Study report number**	: MB-WH-281h-07
1.4	**Job number**	: 281h-07-RBT

2.0 DETAILS OF TEST MATERIAL

2.1	**Name**	: Product named as Natural Sago Hydrogel subsequently referred to as NSH in this report.
2.2	**Test material reference**	: GL-281h-RBT0507
2.3	**Study reference number**	: WH-01-02-07
2.4	**Pkg Lst Batch**	: 6147/54/40/51/52/53
2.5	**Date sample received**	: 21 May 2007
2.6	**Expiry date**	: Not applicable
2.7	**Appearance**	: Solid
2.8	**Colour**	: Transparent
2.9	**Quantity**	: 300 pieces
2.10	**Storage**	: Ambient
2.11	**Condition of use**	: As supplied
2.12	**Dates of study**	: 15th August 2007 to 1st March 2008
2.13	**Date of completion**	: 1st March 2008

3.0 LABORATORY FACILITY

3.1	**Collaboration with**	: Animal Experimental Unit, Unit Makmal Haiwan, Fakulti Perubatan Universiti Malaya, 60603 Lembah Pantai. KUALA LUMPUR

3.3 Project staff

3.3.1 Study Director (1)
Dr. Saadiah Sulaiman
MBBCh (Dub), MMED (UKM)

3.3.2 Study Personnel/Consultant
Assoc. Prof. Dr. Md. Mahmood Ameen Abdulla
BVM&S (Mosul), M.Sc (Mosul), PhD (UPM)

Sharifah Ismail

3.3.3 Quality Assurance Personnel
Arrniza A. Rashid

3.4 Address of correspondence

MEDIAMAL TESTING & CONSULTANCY SERVICES (MTES)
Lot B-G-34, Pangsapuri Sri Penara,
Jalan Sri Permaisuri 1,
Bandar Sri Permaisuri,
Cheras 56000 KUALA LUMPUR.
Tel/Fax: 03-91737022
Tel: 03-91726629

c/o
Fadhilah binti Manap

3.5 Study Timetable

Date of initiation : 15th August 2007
Date of completion : 1st March 2008

3.6 Environment and Husbandry

3.6.1 Species and strain
Sprague Dawley albino rat.

3.6.2 Supplier
Animal Laboratory, Universiti Malaya and Institute Medical Research.

3.6.3 Number of animal and sex
Fifteen Sprague Dawley albino rats; 5 for test group (females), 5 for negative control (females) and 5 for positive control (males).

3.6.4 Body weight at initiation of treatment

Weight:	Test group	: 149 - 159 g
	Negative control group	: 156 - 168 g
	Positive control group	: 182 - 205 g

3.6.5 Housing
Animals were housed in a plastic polypropylene caging with perforated base.

3.6.5.1 Size of cage: Plastic cage internal dimension: 22cm x 15cm x 37cm. Each rat was housed in a separate cage and assigned specific numbers from 1 to 5.

3.6.5.2 Photoperiod
12 hour light/dark cycle.

3.6.6 Diet
Mouse pellet: Gold Coin Brand Animal Feed (Cahaya Suria Sdn Bhd) and Australia pellet.

3.6.7 Water
Filtered tap water *ad libitum* continuously supplied through water dispensing bottles.

3.6.8 Contaminant
There were no known contaminants reasonably expected to be found in the food or water at levels which would interfere with the results of the study.

3.7 Pre-Treatment Procedures

3.7.1 Check for ill health
On arrival, daily and just before commencement of treatment to ensure only healthy animals were used in the study.

3.7.2 Body weight
All animals were weighed on the day just before treatment, once weekly and at termination of study.

3.7.3 Selection and allocation of animal
Rats were selected randomly at the start of the acclimatization period.

3.7.4 Identification
Cages : Labeled with animal number, sex of animal and study reference number.
Animal : Each cage of rat was marked with a color code (permanent marker pen) and given a sequential animal number assigned to study reference number, which constitutes a unique identification system.

4.0 TEST METHOD

4.1 Name of test: Efficacy Assessment of NSH for Wound Healing of Normal Rats

4.2 Objective
To evaluate the efficacy of a test material named as Natural Sago Hydrogel following topical administration on wound of normal rats.

4.3 Material

4.3.1 Test Material: Topical product named Natural Sago Hydrogel (NSH)
4.3.2 Test system
Sprague Dawley albino rats; five for test group (females), 5 for negative control (females) and 5 for positive control (males).

4.4 Preparation of test material
4.4.1 Route of test material administration
The route of test material administration were by topical application. Test material was applied as delivered.

4.4.2 Method of test material administration
The test material (2x2cm) was administered directly on to the designated treatment site.

4.5 Procedure

4.5.1 Preparation and selection of animals

On the day before initiation, the fur of rats were removed by clipping on the dorsal neck by electrical clipper. The rats were anesthetized with local anesthesia, i.e subcutaneous injections of lignocaine 2%. A 1.8-cm diameter circle was drawn with a marker pen on the test area of the dorsum of the neck. A full-thickness circular skin graft with a surface area of 5.66 cm^2 was harvested from the dorsum of each rat using surgical scissors. The full-thickness skin defect was left to heal by secondary intention, and each rat was kept in a separate cage with the defect uncovered.

4.5.2 The rats were divided into 3 groups of 5 rats. The first group was the study group and was treated with NSH topically. The second group was the negative control and left untreated and the third group was the positive control and was treated with Intrasite gel as positive control. All topical application was done once daily until the wound completely healed.

4.5.3 Treatment and observation period

Each rat was observed for improvement of wound daily. The day the wound is completely healed is taken as the end point.

Histopathology

Upon healing, a full thickness biopsy of the healed wound is excised for histopathological examination.

4.6 Method of assessment

1. Clinical assessment
   Mean duration of time taken for complete healing of wound i.e clearance of raw surface.
2. Histological assessment
   Full thickness biopsy of each wound area was excised, stored in formalin and subsequently processed into tissue block. Sections were then made and stained with Hematoxylin and Eosin (H&E) staining for histological assessment.

5.0 DATES OF TEST PROCEDURE

Date of initiation	: 15th August 2007
Date of completion	: 1st March 2008

6.0 SUMMARY OF RESULTS

6.1 A total of 15 normal rats were completed the study, five for test group treated with NSH and five for negative control (left untreated) and five for positive control (treated with Intrasite gel).

6.2 The duration the wound take to heal is as tabulated in Table 1. The group treated with NSH took a significantly shorter duration of time for complete wound healing, i.e 12.4 days compared to 15.8 days for negative control, $p=0.000$ (i.e $p<0.05$). This group also had a shorter duration for complete wound healing compared to positive control that took 12.8 days but the difference was not significant, $p=1.000$ (i.e $p>0.05$). Positive control took a shorter duration for complete wound healing compared to negative control, $p=0.000$ (i.e $p<0.05$).

Table 1: Duration of the time taken for complete wound healing by Group Treated with NSH, compared to Negative Control (untreated) and group Treated with Intrasite gel (Positive Control).

Group	Animal number	Test animal sequences	n	Day of complete healing	No of days wound take to heal	Mean
Test Group (NSH)	rwt11	03-1	5	Day 13	13	12.4
	rwt12	03-2		Day 12	12	
	rwt13	03-3		Day 12	12	
	rwt14	03-4		Day 13	13	
	rwt15	03-5		Day 12	12	
Negative control	rwn01	nc-1	5	Day 17	17	15.8
	rwn02	nc-2		Day 15	15	
	rwn03	nc-3		Day 16	16	
	rwn04	nc-4		Day 16	16	
	rwn05	nc-5		Day 15	15	
Positive control	rwp06	pc-1	5	Day 13	13	12.8
	rwp07	pc-2		Day 14	14	
	rwp08	pc-3		Day 12	12	
	rwp09	pc-4		Day 13	13	
	rwp10	pc-5		Day 12	12	

Table 2: Comparison of p value for each group in normal rats.

Comparison	Actual p value	p value	
NHS vs PC	1.000	p>0.05	Not significant
NHS vs NC	0.000	P<0.001	Significant
PC vs NC	0.000	P<0.001	Significant

6.2.1 PICTURES

1. Picture 1

   Demonstrates the difference in wound healing between rats treated with NSH compared to untreated group (negative control).

   Pictures 1a and 1b:
   At Day 7, group treated with NHS had smaller diameter, 1.2 cm x 1.1 cm compared to those left untreated (negative control), 1.6 cm x 1.1 cm.

   Pictures 1c and 1d:
   Complete wound healing is faster in group treated with NHS - Day 13 compared to the untreated group (negative control) - Day 16 (See Appendix I)

2. Picture 2
   Histopathological examination of wound treated with NSH on normal rats (See Appendix II)

3. Picture 3
   Histopathological examination of wound left untreated (negative control) on normal rats (See Appendix III)

6.3 Histopathology

There was histopathological changes observed in all groups i.e group treated with NSH, untreated group (negative control) and group treated with Intrasite gel (positive control). NSH promotes wound healing activity in this excision wound model study, which is more or less equal to positive control (standard treatment- Intrasite gel). In all NSH treated wounds, the histological appearance of skin showed absence of inflammatory cells, increased collagen formation, fibroblast cells, new blood capillaries (angiogenesis) and re-epithelialization compared to the negative control. Although there were differences between NSH and Intrasite gel treated groups, but this difference was not significant. Granulation tissue of treated animals showed moderate deposition of collagen comparable to Intrasite gel treated animals. Scar area in wound closure was also noted to be smaller with NSH and Intrasite gel compared to negative control.

7.0 CONCLUSION

NSH is effective in promoting wound healing in normal rats, significantly shortens the duration of wound healing, and significantly promotes wound healing activity in the excised wound model. It is as effective as Intrasite gel in promoting wound healing in normal rat.

8.0 VERIFICATION

We the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

.....

Dr. Saadiah Sulaiman
Study Director 1

26/6/09
.....
Date

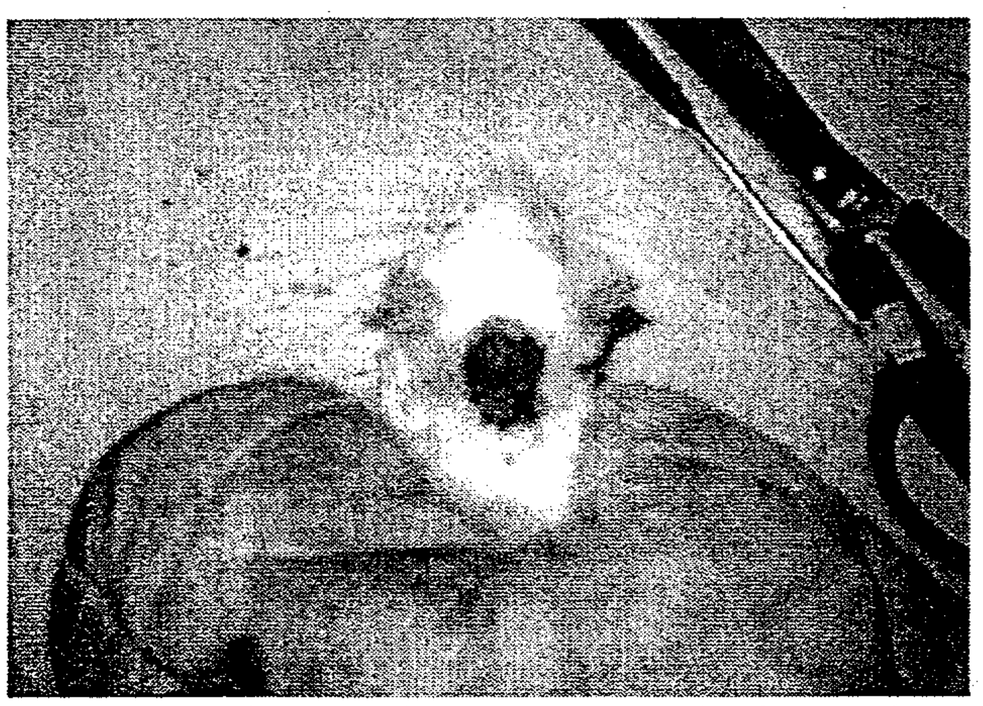
.....

Assoc. Prof. Dr. Md. Mahmood Ameen Abdulla
Study Director 2

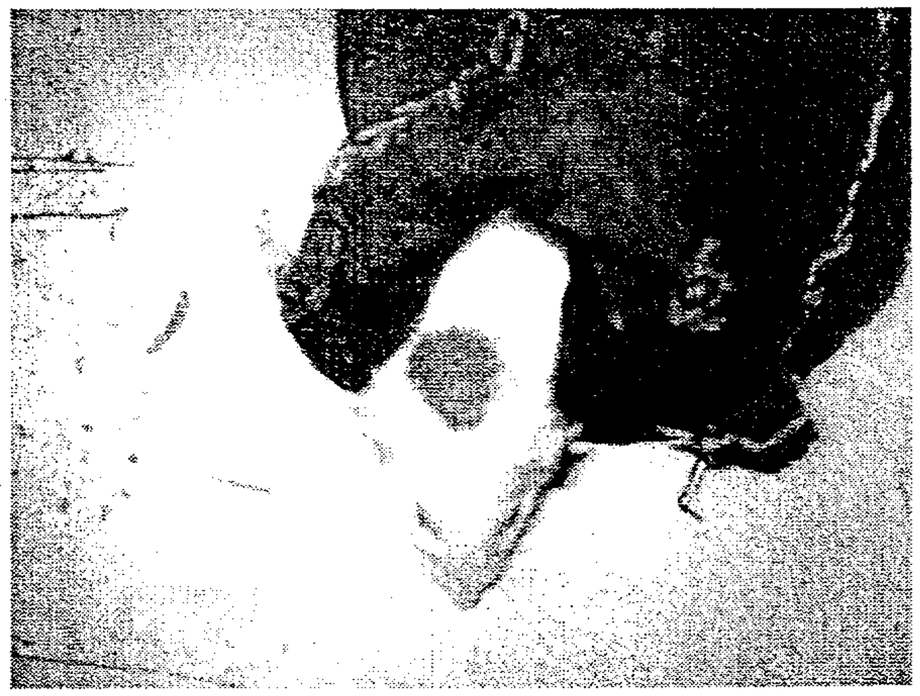
26/6/09
.....
Date

Appendix I

Pictures 1:
Demonstrates the difference in wound healing between rats treated with NSH, compared to rats from untreated group (negative control).

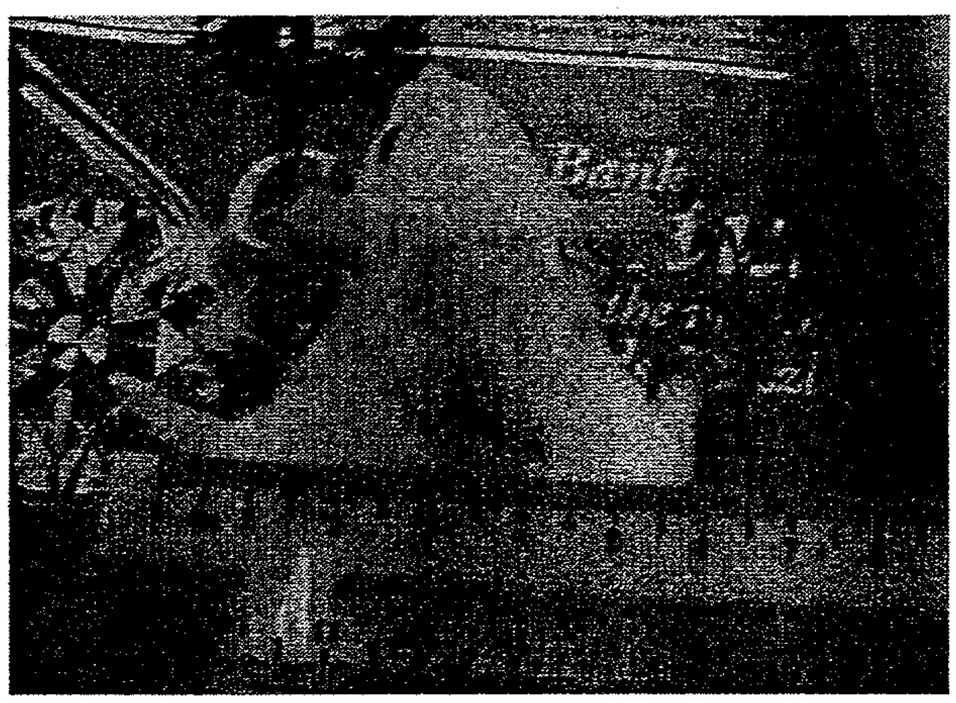


Picture 1a: Diameter (1.2 cm x 1.1 cm) of wound on Day 7 (test group).



Picture 1b: Diameter (1.6 cm x 1.1 cm) of wound on Day 7 (negative control).

Pictures 1a and 1b at Day 7:
Demonstrate that a rat from the group treated with NHS had smaller diameter, 1.2 cm x 1.1 cm compared to a rat from untreated group (negative control), 1.6 cm x 1.1 cm.



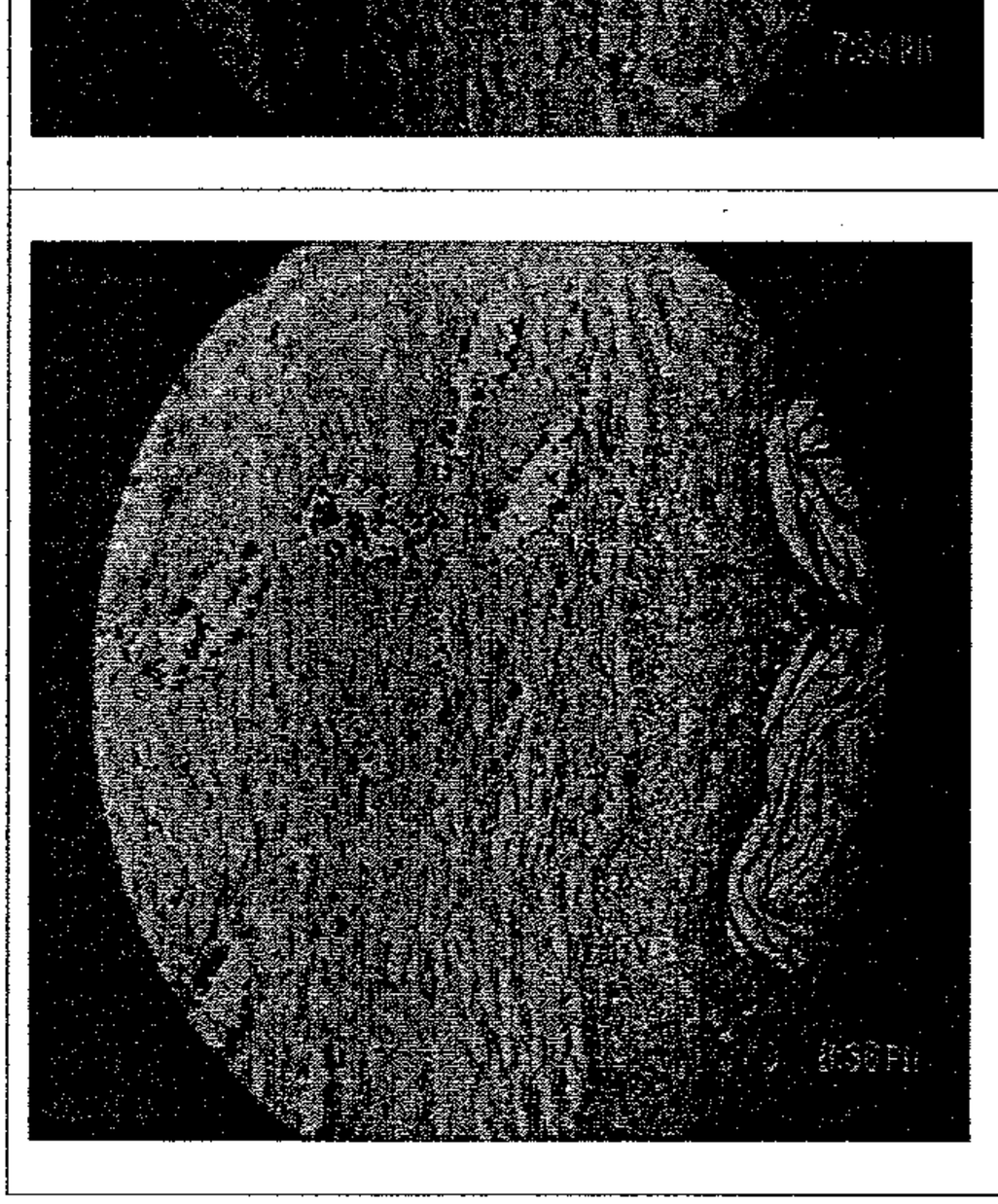
Picture 1c: Day (Day 13) of complete wound healing (test group).

Picture 1d: Day (Day 16) of complete wound healing (negative control).

Pictures 1c and 1d:
Complete wound healing is faster in a rat from the group treated with NSH - Day 13 compared to the rat from untreated group (negative control) - Day 16.

Appendix II

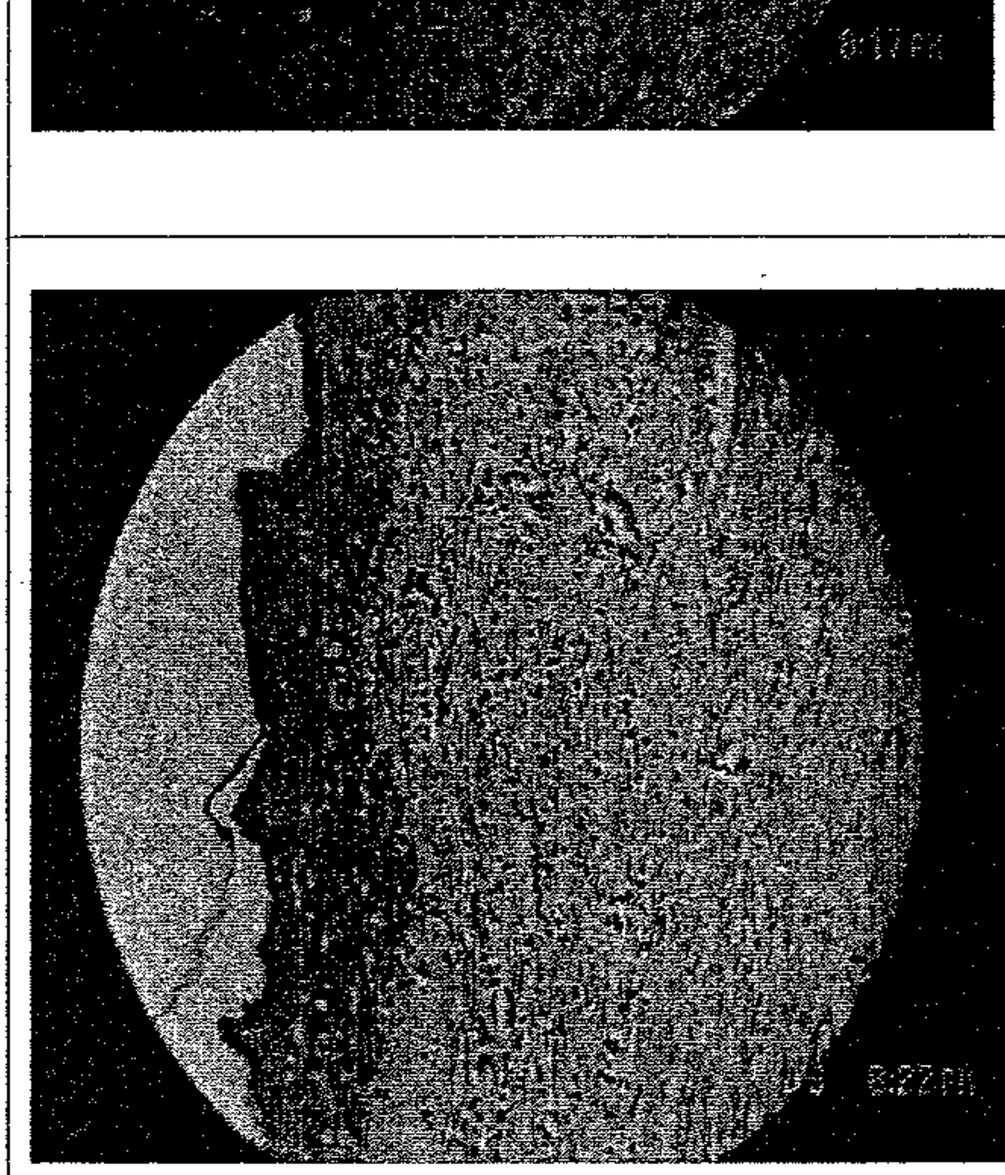
Picture 2:
Histopathological Examination of Wound Treated with NSH on Normal Rats.



Large granulation tissue, more collagen, angiogenesis and re-epithelization, and more fibroblast and myofibroblasts and no inflammatory cells compared to untreated wounds.
Wound enclosure are fast and regular, and less scaring.

Appendix III

Picture 3:
Histopathological Examination of Wound left Untreated (Negative Control) on Normal Rats.



Less granulation tissue, collagen, angiogenesis and re-epithelization, and less fibroblast and myofibroblasts and more inflammatory cells compared to treated wounds with NSH.
Wound enclosure are moderate and regular, and moderate scaring.