



RURAL INDUSTRIES  
RESEARCH & DEVELOPMENT CORPORATION

# **Determining the efficacy of emu oil in wound healing and cellular regeneration**

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A report for the  
Rural Industries Research & Development Corporation  
by  
John M. Snowden, Michael Roberts and Sheree Cross

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*'Determining the efficacy of emu oil in wound healing and cellular regeneration'*

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**Researcher Contact Details**

Dr John M. Snowden  
Agriculture Western Australia  
3 Baron-Hay Court  
SOUTH PERTH WA 6151

Phone: (08) 9368 3555  
Fax: (08) 9474 2479  
E-mail: [jsnowden@agric.wa.gov.au](mailto:jsnowden@agric.wa.gov.au)

**RIRDC Contact Details**

Rural Industries Research and Development Corporation  
Level 1, AMA House  
42 Macquarie Street  
BARTON ACT 2600

PO Box 4776  
KINGSTON ACT 2604

Phone: 06 272 4539  
Fax: 06 272 5877  
E-mail: [rirdc@netinfo.com.au](mailto:rirdc@netinfo.com.au)  
Internet: <http://www.dpie.gov.au/rirdc>

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## Foreword

High prices are paid for emu fat because emu oil is believed to have therapeutic activity. To expand the therapeutic uses of emu oil registration as an active ingredient under the Therapeutic Goods Act is required. The first step towards registration is to demonstrate the efficacy of emu oil in a scientifically acceptable manner.

Rural Industries Research and Development Council is supporting a number of projects investigating the potential therapeutic applications of emu oil. These are, in order of priority, the anti-inflammatory properties of emu oil, the effects of emu oil on wound healing and cellular regeneration, the anti-viral/anti-bacterial activity and the cholesterol lowering cardiovascular effects of emu oil.

This publication describes a study into the effects of the topical application of emu oil on the healing of full depth excisional wounds.

The project is part of RIRDCs New Animal Industries Program which aims to accelerate the development of viable new animal product industries.

Peter Core  
MANAGING DIRECTOR  
RURAL INDUSTRIES RESEARCH AND DEVELOPMENT CORPORATION



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## Executive Summary

In the past few years emu production has become a multimillion dollar industry in this country. Until recently, the profitability of this new industry has relied largely on breeding and selling chicks into an expanding industry. However, it is apparent that in the near future the industry will have to depend on the sale of emu products. The products include meat, skins, fat (oil) and feathers. Based on the current and predicted prices for these products, it would seem likely that emu farming will only remain profitable if the very high price currently paid for the fat is maintained. Until recently, the price obtained for emu fat was between \$20 and \$25 per kilo, compared to around 20 cents per kilo for fat from other animal sources. However, this will drop rapidly, as increasing supplies become available, unless high value end uses are established.

To expand the therapeutic use of emu oil, registration as an active ingredient under the Therapeutic Goods Act is required. The first step towards registration is to demonstrate the efficacy of emu oil in a scientifically acceptable manner.

To date the evidence for the efficacy of emu oil in wound healing been largely anecdotal, e.g. used by aboriginals for centuries for treating burns and emus wounds heal very rapidly. The actual evidence available to support the proposal that emu oil is efficacious in wound healing and cellular regeneration is sparse and unconvincing.

Recently, preparations of emu oil have been shown, using appropriate animal models, to possess significant anti-inflammatory activity. Since all currently available anti-inflammatory drugs impede wound healing it was important to assess the effects of emu oil on wound healing.

Excisional wounds are wounds where a considerable area of dermis is removed. These wounds heal by a combination of contraction, the inward movement of the wound margins, and epithelialisation, the outward growth of epithelium from the wound margins. Healing involves the rapid development and subsequent removal of relatively large quantities of granulation tissue, a fibroblastic tissue. Full-thickness wounds in most experimental animals, including rats, contract rapidly and, in terms of area covered, contraction makes the major contribution to closure. This form of healing is also relevant to the healing of burns particularly severe burns, one of the most common causes of severe trauma to young children.

The objectives of the study were to:

1. By using an appropriate animal model(s), to determine if emu oil is efficacious in the treatment of excisional wounds.
2. To recommend areas for further research and development.

In this study, the rates of contraction of full-thickness wounds in rats treated topically with one of four preparations of emu oils or liquid paraffin, as a control, were measured by daily tracing the wounds. The four preparations of emu oil used had varying degrees of anti-inflammatory activity in both the adjuvant induced polyarthritis and carrageenan induced oedema rat models.

After tracing, fresh oil was applied and the wounds redressed. The wounds were monitored for 12 consecutive days.

The rates of contraction were calculated using the procedure described by Snowden (Snowden, J.M. Wound contraction: A quantitative interpretation. *Aust. J. Exp. Biol. Med. Sci.* **59**: 217, 1981). With dressed wounds three phases of healing are observed. A latent or lag phase (phase I) during which little or no contraction occurs. This phase is generally quite short. This is followed by a period of rapid contraction. During this phase the wound margins move at a constant linear rate perpendicular to the wound edge and the rate of contraction is proportional to the slope of the plot of the square root of wound area versus time. Contraction then ceases and the wound closes by epithelialization. The results obtained on days 1 through 9 were used to calculate the rate of contraction shown in Table 1.

**Table 1. Analysis of wound contraction data**

Group	Rate of contraction (mean $\pm$ SEM) (mm/day)	r*
Emu oil 1	1.22 $\pm$ 0.06	0.988
Emu oil 2	1.26 $\pm$ 0.07	0.971
Emu oil 3	1.26 $\pm$ 0.03	0.987
Emu oil 4	1.20 $\pm$ 0.06	0.978
Paraffin	1.22 $\pm$ 0.03	0.985

\* Average linear regression coefficient of the plots of (wound area)<sup>1/2</sup> versus time.

It can be seen (Table 1) that, with all groups of animals, there was good agreement between the experimentally observed change in wound area due to contraction and that expected on the basis of a constant linear rate of movement of the wound margins. Also that the rates of contraction were the same for all groups. Analysis of the results using ANOVA gave a P of 0.95.

None of the emu oils caused any adverse effects and all animals showed healthy granulation tissue throughout the contractile phase. There was insufficient epithelialization to make any quantitative comparisons but visually there were no differences.

From one point of view, the failure to demonstrate any efficacy of emu oil is disappointing. However, the demonstration of no adverse effects will be of considerable value in presenting the case to the relevant Ethics Committees if it is decided that the anti-inflammatory study should proceed to human clinical trials. Such trials will be required before Therapeutic Goods Act registration can be obtained.

The following recommendations are made:

1. The investigation into the effects of emu oil on wound healing be discontinued.
2. A small sum of money (\$5,000) be allocated to test the actual preparations of oil that are to be used in clinical trials.

3. Any money available for emu oil research would be better spent in other areas of research, e.g. anti-inflammatory activity and/or the use of emu oil as a transdermal carrier.



# Introduction

In the past few years emu production has become a multimillion dollar industry in this country. Until recently, the profitability of this new industry has relied largely on breeding and selling chicks into an expanding industry. However, it is apparent that in the near future the industry will have to depend on the sale of emu products. The products include meat, skins, fat (oil) and feathers. Based on the current and predicted prices for these products, it would seem likely that emu farming will only remain profitable if the very high price currently paid for the fat is maintained. Until recently, the price obtained for emu fat was between \$20 and \$25 per kilo, compared to around 20 cents per kilo for fat from other animal sources. However, this will drop rapidly, as increasing supplies become available, unless high value end uses are established.

High prices are paid for emu fat because emu oil is believed to have therapeutic and cosmetic applications. It is probable that in the near future the amount of emu oil required for cosmetics production will be limited. Reasons for this include the small percentage of oil in the products, the cost of production (around \$2 per kilo for rendering and around \$5 per kilo for refining) and there is a lack of any documentary evidence that emu oil performs any better than a range of cheaper alternatives. Furthermore, particularly in Australia, there is a move away from cosmetics that contain animal products (David Stacy, Orion Laboratories, WA).

To expand the therapeutic use of emu oil, registration as an active ingredient under the Therapeutic Goods Act is required. The first step towards registration is to demonstrate the efficacy of emu oil in a scientifically acceptable manner.

Rural Industries Research and Development Council (RIRDC) invited Agriculture Western Australia to facilitate a national emu oil research and development project over the period 1996/97 to 1998/99. As part of the facilitation, Agriculture Western Australia recently organised a meeting, sponsored by RIRDC, of industry representatives and researchers with expertise and interest in emu oil research to develop a plan for a national emu oil research development program to be funded by RIRDC. The participants identified the following as areas requiring further research, the areas are listed in order of priority:

1. Anti-inflammatory properties of emu oil.
2. The effects of emu oil on wound healing and cellular regeneration.
3. Anti-viral/anti-bacterial activity.
4. Cholesterol lowering cardiovascular effects of emu oil.

If efficacy of emu oil can be established, it is proposed to investigate the effects of a variety of factors, such as rendering and refining procedures, the diet of the birds and the source of the oil, on the efficacy of the oil

To date the evidence for the efficacy of emu oil in wound healing has been largely anecdotal, e.g. used by aboriginals for centuries for treating burns and emu wounds heal very rapidly.

The actual evidence available to support the proposal that emu oil is efficacious in wound healing and cellular regeneration is sparse and unconvincing.

A United States Patent (1) includes examples that are claimed to demonstrate that emu oil prevents scarring. The examples are, however, only anecdotal and there is no actual claim made for this application.

In private studies, funded by Mount Romance, the effects of emu oil on the proliferation of cells in culture were examined. In the first study, the addition of emu oil was found to significantly ( $p < 0.01$ ) increase, by up to 34%, the rate of proliferation of a fibroblast cell line. The effect was shown to be dose dependant. In the subsequent study, the addition of emu oil was found to have no effect on the rate of proliferation of keratinocytes.

In an article in a drug and cosmetic industry magazine (2) there is a report of study performed at Boston University Medical Centre using a mouse model for evaluating the biological activity of new substances on skin and hair growth. Two groups of mice had their hair removed by depilation with a wax rosin. After 24 hours one group received topically 0.1 mL of emu oil and the other 0.1 mL corn oil. The oils were then applied daily for the next 14 days. At the end of the studies samples were collected for histological examination and for the measurement of thymidine incorporation. The samples obtained from animals treated with emu oil incorporated approximately 29% more thymidine than those from the corn oil treated animals. It is not stated whether this difference was statistically significant or not.

Recently, preparations of emu oil have been shown, using appropriate animal models, to possess significant anti-inflammatory activity (3 & 4). Since all currently available anti-inflammatory drugs impede wound healing it was important to assess the effects of emu oil on wound healing.

The first priority was to decide which type of wounds to study. Basically, wounds can be divided into superficial and deep wounds. Superficial wounds generally heal rapidly and without scarring and are of little interest in the present context.

Deep wounds again fall basically into incisional and excisional wounds. Incisional wounds are where the wound edges are drawn together to heal and are said to heal by primary intention. In general incisional wounds heal without complications and result in minimal scarring. Excisional wounds are wounds where a considerable area of dermis is removed. These wounds heal by a combination of contraction, the inward movement of the wound margins, and epithelialisation, the outward growth of epithelium from the wound margins. Healing involves the rapid development and subsequent removal of relatively large quantities of granulation tissue, a fibroblastic tissue. Full-thickness wounds in most experimental animals, including rats, contract rapidly and, in terms of area covered, makes the major contribution to closure. This form of healing is also relevant to the healing of burns particularly severe burns, one of the most common causes of severe trauma to young children.

Excisional wounds are the most appropriate form of wound to use in an investigation of efficacy of emu oil in wound healing. Difficulties associated with using such wounds are that animals require care and treatment for a relatively long time (2 to 3 weeks). Also, this type of wounding can be visually upsetting to the public and so a strong case needs to be put to gain consent from animal ethics committees. The advantages are that information on the effects of

oil on a number of processes is obtained at the same time, the results are quantitative and in the correct context, a strong case for the justification of this type of research can be made.



## **Objectives**

1. By using an appropriate animal model(s), to determine if emu oil is efficacious in the treatment of excisional wounds.
2. To recommend areas for further research and development.



## Methodology

Emu oils were prepared from the fat obtained from two birds raised by Agriculture Western Australia. The fat samples used contained two thirds subcutaneous fat and one third gut fat. Oil samples were prepared by heating at 50, 60, 80 100°C and designated JS-50, JS-60, JS-80 and JS-100 respectively. The anti-inflammatory activities of the preparations were assessed using both the adjuvant induced poly-arthritis and carrageenan induced oedema rat models. Liquid paraffin was purchased from Sigma Chemical Company, Sydney.

The wound healing studies were performed at the Department of Medicine, University of Queensland, as a consultantancy to Agriculture Western Australia. The group already had the approval of the Universities Animal Ethics Committee to perform this type of study.

Male Wistar rats,  $281 \pm 8$  g, were anaesthetised with pentobarbitone sodium (60 mg/kg i.p.), their lower back and left flank clipped with electric clippers and depilated with commercial hair removal cream (Nair<sup>®</sup>). After thoroughly cleaning with water, swabbing with hibitane and drying the area, a template measuring 15 x 15 mm was on the skin and outlined using a fine felt-tipped pen.

Full-thickness wounds were produced by excising the skin and subcutaneous tissue down to the level of the muscle fascia using surgical scissors and forceps. Wounds were immediately dosed with 200  $\mu$ L of emu oil or liquid paraffin, dressed with semi-permeable transparent self-adhesive dressings (Opsite<sup>®</sup>, Smith and Nephew), secured at a distance of over 10 mm from the wound edge with spots of cyanoacrylate adhesive (Supa glue gel). Wound areas were traced, in triplicate, onto acetate strips before the animals were then returned to single housing boxes. Dressings were changed, emu oil or liquid paraffin topically applied and the wound traced, in triplicate, at approximately 24 hour intervals with the animals lightly anaesthetised with Forthane<sup>®</sup> inhalation anaesthetic (Abbott). The acetate tracing strips were photocopied, normal size, and the areas of the wounds determined using a computerised digitiser.

The rates of contraction were calculated using the procedure described by Snowden (5). With dressed wounds three phases of healing are observed. A latent or lag phase (Phase I) during which little or no contraction occurs. This phase is generally quite short. This is followed by a period of rapid contraction (Phase II). During this phase the wound margins move at a constant linear rate perpendicular to the wound edge and the rate of contraction is proportional to the slope of the plot of the square root of wound area versus time. Contraction then ceases and the wound closes by epithelialization (Phase III). A typical plot of the square root of wound area versus time is shown in Figure 1.

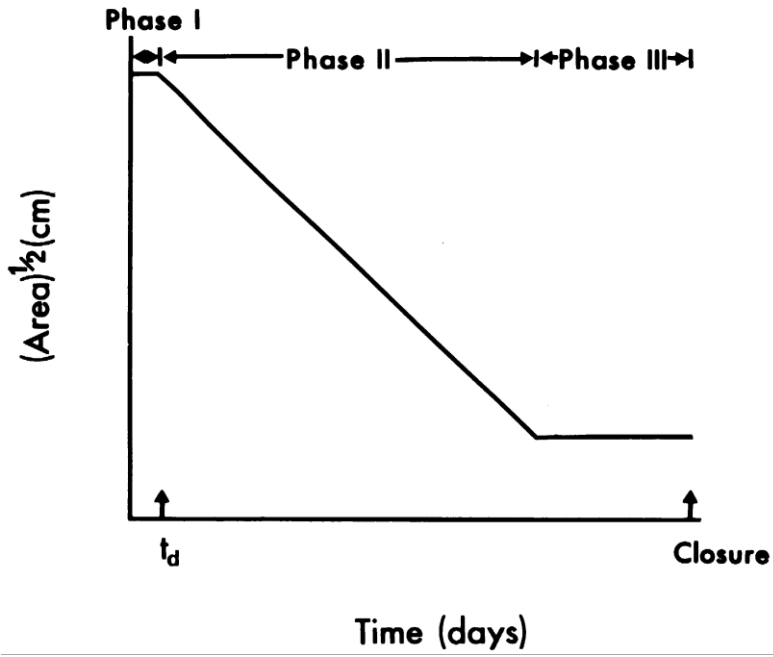


Figure 1. A typical plot of (wound area)<sup>1/2</sup> versus time.

The results obtained on days 1 through 9 were used to calculate the rate of contraction.

## Results

The following Tables (1A-1E) show the wound areas for individual animals at the various times. The areas are given in square millimetres.

**Table 1A. JS-50 treated group**

Day	Rat number				
	1	6	11	16	21
0	239	241	258	271	241
1	208	142	212	231	191
2	149	99	160	199	142
3	143	74	126	150	133
4	87	50	103	119	107
5	53	36	69	82	84
6	36	45	71	68	54
7	23	33	51	61	38
8	18	26	30	64	21
9	13	16	23	39	21
10	10	7	19	33	19.5
11	9	8	11	21	13
12	0	0	0	15	8

**Table 1B. JS-60 treated group**

Day	Rat number				
	2	7	12	17	22
0	400	216	353	287	268
1	274	170	244	245	216
2	239	189	179	220	130
3	217	104	135	198	97
4	215	92	137	175	75
5	137	73	103	139	63
6	102	68	82	95	39
7	81	66	59	65	40
8	64	44	39	61	24
9	38	29	32	40	22
10	37	31	26	34	18
11	32	24	13	21	9
12	30	24	13	17	0

**Table 1C. JS-80 treated group**

Day	Rat number				
	3	8	13	18	23
0	254	280	273	254	279
1	180	199	202	195	224
2	112	155	191	206	178
3	94	121	128	151	153
4	72	112	103	108	127
5	45	77	79	92	110
6	40	53	65	67	78
7	27	28	41	62	55
8	17	26	34	44	40
9	11	22	26	33	33
10	10	7	18	20	20
11	7	2	12	7	12
12	5	0	11	10	14

**Table 1D. JS-100 treated group**

Day	Rat number				
	4	9	14	19	24
0	267	256	260	275	279
1	173	190	195	221	214
2	180	126	174	214	192
3	174	136	119	214	149
4	134	114	121	225	119
5	106	64	71	174	85
6	92	42	43	126	61
7	54	27	34	85	53
8	45	22	33	73	33
9	29	18	26	62	28
10	26	15	19	64	18
11	13	7	8	37	5
12	12	0	6	29	0

**Table 1D. Liquid paraffin treated group**

Day	Rat number				
	5	10	15	20	25
0	252	223	311	262	256
1	149	162	232	234	242
2	153	124	222	161	251
3	139	101	197	152	191
4	101	76	178	132	165
5	61	47	138	82	155
6	50	45	95	78	129
7	38	24	68	44	87
8	31	12	56	41	57
9	17	8	41	34	35
10	20	11	34	28	33
11	12	7	34	19	23
12	13	0	29	28	17

The results of fitting linear regressions to the plots of (wound area)<sup>1/2</sup> versus time are summarised in Table 2.

**Table 2. Analysis of wound contraction data**

Group	Rate of contraction (mean ± SEM) (mm/day)	r*
JS-50	1.22 ± 0.06	0.988
JS-60	1.26 ± 0.07	0.971
JS-80	1.26 ± 0.03	0.987
JS-100	1.20 ± 0.06	0.978
Paraffin	1.22 ± 0.03	0.985

\* Average linear regression coefficient of the plots of (wound area)<sup>1/2</sup> versus time.

It can be seen (Table 2) that, with all groups of animals, there was good agreement between the experimentally observed change in wound area due to contraction and that expected on the basis of a constant linear rate of movement of the wound margins. Also that the rates of contraction were the same for all groups. Analysis of the results using ANOVA gave a P of 0.95.

None of the emu oils caused any adverse effects and all animals showed healthy granulation tissue throughout the contractile phase. There was insufficient epithelialization to make any quantitative comparisons but visually there were no differences.



## Discussion

In this study the topical application of emu oil did not significantly alter the rates of contraction of full thickness excisional wounds in rats. Also, the application of emu oil was found not to cause any adverse effects. The four preparations of emu oil used had varying degrees of anti-inflammatory activity in both the adjuvant induced polyarthritis and carrageenan induced oedema rat models.

From one point of view, the failure to demonstrate any efficacy of emu oil is disappointing. However, the demonstration of no adverse effects will be of considerable value in presenting the case to the relevant Ethics Committees if it is decided that the anti-inflammatory study should proceed to human clinical trials.

The results of this study do not preclude the possibility that emu oil may have some application in the treatment of superficial wounds (burns) where the ability of emu oil to reduce inflammation could be beneficial, e.g. relieve pain. However, we are not aware of any animal model that is suitable for scientifically assessing such effects. Similarly, there are no suitable models to examine the effects of emu oil on scarring and there is insufficient reason to justify the expense of a quantitative epithelialization study.

In view of the lack of any effect of emu oil, there would seem little, if any point, in continuing this line of research at this time. However, it is recommended that a relatively small quantity of funds (\$5,000) be allocated to test the actual preparations that are to be used in clinical trials

If there was the desire to continue on with work on cellular regeneration the only reasonable option would seem to be *invitro* tissue culture studies. Although such studies would probably contribute little to obtaining Therapeutic Goods Act registration of emu oil.



## Implications

The demonstration that there were no adverse effects will facilitate obtaining permission from the relevant Ethics Committees to perform human clinical trials on the anti-inflammatory activity of emu oil. Such trials will be required before Therapeutic Goods Act registration can be obtained.



## Recommendations

1. The investigation into the effects of emu oil on wound healing be discontinued.
2. A small sum of money (\$5,000) be allocated to test the actual preparations of oil that are to be used in clinical trials.
3. Any money available for emu oil research would be better spent in other areas of research, e.g. anti-inflammatory activity and/or the use of emu oil as a transdermal carrier.



## Intellectual property

None.



## **Communications strategy**

The results of this study will be published with the results of the anti-inflammatory studies.



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