OP-036 [Apitherapy]
Antibacterial efficacy of Apis honey collected from different Apiaries and its effect on Sprague Dawley rats

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With the prevalence of antibiotic-resistant bacteria, honey is increasingly valued for its antibacterial activity. Honey is a traditional topical treatment used for infected wounds and can be effective on antibiotic resistant strains of bacteria. Their antibacterial activity varies with origin and processes. Honey samples collected from local market were analyzed to test their antibacterial effect against B.subtilis and E.coli. The specific growth rate (μ) of the bacteria was reduced in the presence of honey samples. Commercial honey collected from the local supermarket showed less antibacterial activity than raw honey samples. The efficacy of honey, which showed highest antibacterial activity on the healing of wounds in Sprague Dawley rats was investigated on the basis of biophysical and biochemical changes. Equidimensional full-thickness excision wounds were created on the back of each animal and treated by topical application of honey. Thirty six rats were divided into six groups of six rats each namely, Group I(25% honey treatment), Group II(50% honey treatment), Group III(75% honey treatment), Group IV (100% honey treatment) and Group V (Saline water application). Control animals received commercial antibiotics (neomycin ointment). The rate of wound contraction was superior in animals treated with the honey along with a significant increase in the level of serum albumin as compared to the other groups. The histopathological studies revealed confirmatory evidence for improved healing activity by the honey as evidenced by improved granulation tissue formation and enhanced epithelialisation in group IV as compared to that of groups V and VI.

Antibacterial efficacy of *Apis* honey collected from different apiaries and its effect on Sprague Dawley rats.

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APITHERAPY ASSOCIATION OF INDIA
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Honey

Honey is valued not only as a natural sweetener but also for its traditional and scientifically supported medicinal benefits. These benefits are largely attributed to its properties as an

- **anti-inflammatory**,
- **antioxidant**, and
- **antibacterial** agent.

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Sample

Four Unprocessed raw honey samples

One Processed raw honey sample

Methodology

Sprague Dawley Rat
(150-180g)

Six in a group acclimatization period of 7 days to the laboratory environment

Wound creation:

Rate of Contraction:

Collection of Granulation tissue:

Biochemical analysis:

Antimicrobial Activity:

The work was carried out in CPCSEA approved Animal House of SASTRA University , Thanjavur , Tamil Nadu, India.

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Wound Creation

- Six groups of six rats each were taken for studies.
- The dorsal side of the rat was shaved with razor blade. Using a rod of 2cm diameter, which was heated to 80-85°C burn wounds were created by exposing dorsal side of rats for 20 seconds.
- After 24hrs dead tissues were excised using surgical blade, and the wound site was sterilized by spirit

Group-I treated with 25%(w/w) honey

Group-II treated with 50%(w/w) honey

Group-III treated with 75%(w/w) honey

Group-III treated with 100%(w/w) honey

Group V treated with 5% saline water

Group VI treated with commercial antibiotics (neomycin).

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All the rats were given regular feed and water

Rats kept under observation for up to 14 days giving regular dressing with honey.

After the removal of the dead tissues the initial wound size was measured.

Two animals from each group were sacrificed on every 5^{th}, 10^{th} and 15^{th} day and blood was collected and serum was separated.

This wound tissue was deserted out and washed in ice-cold saline and was used for various experiments.

Histopathological Evaluation of Wound Healing Activity

The different stages of wound healing observed were categorized as follows:

1. Inflammatory Phase

- presence of thrombus;
- moderate to marked inflammatory cells,
- predominantly the polymorphonuclear cells
- moderate mononuclear cells;
- mild to moderate fibrinous exudate and
- **absence of reepithelialization**

.

2. Early fibroblastic phase

- presence of thrombus;
- minimal to moderate inflammatory cells,
- moderate to marked vascular granulation tissue,
- minimal to moderate fibrous granulation tissue,
- a minimal fibrinous exudate and
- **initiation of reepithelialization**

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3. Late fibroblastic phase

- presence of thrombus;
- minimal number of inflammatory cells,
- minimal to marked fibrous granulation tissue,
- minimal to moderate vascular granulation tissue and
- **. minimal to moderate reepithelializatio n**

4. Wound contraction phase

- reduction of the wound space,
- **complete reepithelialization**
- abundance of fibrous granulation tissue,
- minimal / scanty vascular granulation tissue and
- absence of thrombus.

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Table 4 : Minimum and Maximum Inhibitory Concentrations in different honey samples.

- Honey's showed 75% maximum inhibitory concentration against *B.subtilis* and more than 75% against *E.coli* while all other honeys showed minimum inhibitory concentrations of less than or equal to 25% and maximum inhibitory concentrations of more than 75% against the two bacterial strains.

	Concentration % (wt/vol)									
	AC1		AC2		AP		AC4		AC5	
Bacteria	**Xmin**	**Xmax**	**Xmin**	**Xmax**	**Xmin**	**Xmax**	**Xmin**	**Xmax**	**Xmin**	**Xmax**
E.*coli*	≤25	>75	25	>75	25	**>75**	25	>75	<25	>75
B.*subtilis*	25	>75	25-50	>75	25	**75**	25	>75	25	>75

Table 5: Individual Animal Scoring

Animal No.a	Inflammatory cells	Reepith-elialisation	Granulation Tissue		Fibrinous Exudate	Thrombus	Wound Contraction	Inference
			Vascular	Fibrous				
FY 1	++++	-	+	-	+++	+++	-	Early fibroblastic phase
FY 2	++++	-	+	-	+++	+++	-	Early fibroblastic phase
FY 3	+	+	+++	-	+	+	-	Late fibroblastic phase
FY 4	++++	-	++	-	+++	++	-	Early fibroblastic phase
FY 5	++++	-	+	-	+++++	++++	-	Late Inflammatory phase
FY6	++++	-	+	-	+++	+++	-	Early fibroblastic phase
FY 7	+	+	+++	-	+	+	-	Late fibroblastic phase
FY 8	+	+	++++	-	+	+	-	Late fibroblastic phase
FY 9	++	+	++++	-	+	+	-	Late fibroblastic phase
FY 10	++	+	++++	-	+	+	-	Late fibroblastic phase
FY 11	++	+	++++	-	+	+	-	Late fibroblastic phase
FY 12	+	++++++	+++++	+	-	+	+	Wound contraction phase
FY 13	++	+++	++++	-	+	+	-	Late fibroblastic phase
FY 14	+	+++++	++++	+	-	+	+	Wound contraction phase
FY 15	+	++++++	+++++	+	-	+	+	Wound contraction

FX 1	+++++	-	+	-	+++++	++++	-	Inflammatory Phase
FX 2	+++++	-	+	-	+++++	++++	-	Inflammatory Phase
FX 3	+++++	-	+	-	+++++	++++	-	Inflammatory Phase
FX 4	+++++	-	+	-	+++++	++++	-	Inflammatory Phase
FX 5	++++	-	+++	-	+++	+++	-	Early fibroblastic phase
FX 6	+	+	+++	-	-	+	-	Late fibroblastic phase
FX 7	+	+	+++	-	+	+	-	Late fibroblastic phase
FX 8	+	+	++++	-	+	-	-	Late fibroblastic phase
FX 9	++	++	++++	-	+	+	-	Late fibroblastic phase
FX 10	++	+	++++	-	+	+	-	Late fibroblastic phase
FX 11	+	+++++	++++	+	-	+	+	Wound contraction phase
FX 12	+	+++++	++++	+	-	+	+	Wound contraction phase
FX 13	+	+++++	++++	+	-	+	+	Wound contraction phase
FX 14	+	+++++	++++	+	-	-	+	Wound contraction phase
FX 15	+++	+++	++++	-	+	+++	-	Late fibroblastic phase

Table 6: Details of animal scoring

S. No	Grade	Observation
1.	+	Minimal
2.	++	Mild
3.	+++	Moderate
4.	++++	Marked
5.	+++++	Massive /Complete

Stage	Score
Inflammatory phase	1
Early fibroblastic phase	2
Late fibroblastic phase	3
Wound contraction phase	4

Summary of Histopathology results

Table 7a

S. No.	Group	Animal No.	Individual Score		Total Score
1.	**Group I (on 5th Day)**	2	1	2	3
2.	**Group II (on 5th Day)**	2	1	2	3
3.	**Group 1II (on 5th Day)**	2	1	3	4
4.	**Group IV (on 5th Day)**	2	1	2	3
5.	**Group V(on 5th Day)**	2	1	2	3
6.	**Group VI (on 5h Day)**	2	2	1	3

Summary of Histopathology results

Table 7b

S. No.	Group	Animal No.	Individual Score		Total Score
1.	**Group I (on 10th Day)**	2	3	3	6
2	**Group II (on 10th Day)**	2	3	3	6
3.	**Group III (on 10th Day)**	2	3	3	6
4.	**Group IV (on 10th Day)**	2	3	3	6
5.	**Group V (on 10th Day)**	2	2	2	4
6.	**Group VI (on 10h Day)**	2	3	3	6

Summary of Histopathology results

Table 7c

S. No.`	Group	Animal No.	Individual Score		Total Score
1.	Group I (on 15th Day)	2	4	3	7
2.	Group II (on 15th Day)	2	3	4	7
3.	Group 1II (on 15th Day)	2	4	4	8
4.	Group IV (on 15th Day)	2	4	4	8
5.	Group V(on 15th Day)	2	4	3	7
6.	Group VI (on 15h Day)	2	4	3	7

GROUP I

The skin sections of group I revealed moderate amount of fibrous granulation tissue in four out of eight rats.

In the remaining sections, vascular granulation tissue i.e., early stage of the healing process was evident.

Complete epithelialization was found in only one animal of this group.

GROUP II

The skin sections revealed moderate amount of fibrous granulation tissue and the remaining three rats revealed vascular granulation tissue with angiogenesis showing macrophages and lymphocytes in the process of remodeling of blood vessels).

Complete epithelialisation was evident in three rats and partial epithelialisation in two rats, whereas ulcers were still present in the remaining three rats.

GROUP III

This group revealed a moderate amount of fibrous granulation tissue and in the remaining one rat vascular granulation tissue was found.

The granulation tissue showed areas of marked angiogenesis as evidenced by numerous proliferating blood vessels forming interconnected network.

Complete epithelialisation was found in three rats.

GROUP IV

Fibrous granulation tissue was evident .
Vascular granulation tissue of varying grades with numerous proliferating blood vessels was observed in one rat.
Complete epithelialisation was observed in four rats. However, partial epithelialisation was evident in the remaining four rats .

Table 8:The rate of wound contraction on different days in cm

GROUPS	5TH DAY	10TH DAY	15TH DAY
I	1.9±.2	1.2±..22	0.2±.12
II	1.8±.27	1.2±1.7	0.2±.5
III	1.8±.31	1.15±.18	**0.1±.22**
1V	1.6±.23	1.1±.12	0.1±.27
V	1.9±.16	1.6±..18	0.4±.17
VI	1.75±.27	1.2±.14	0.1±.27

Fig 1: Rate of wound contraction in cm

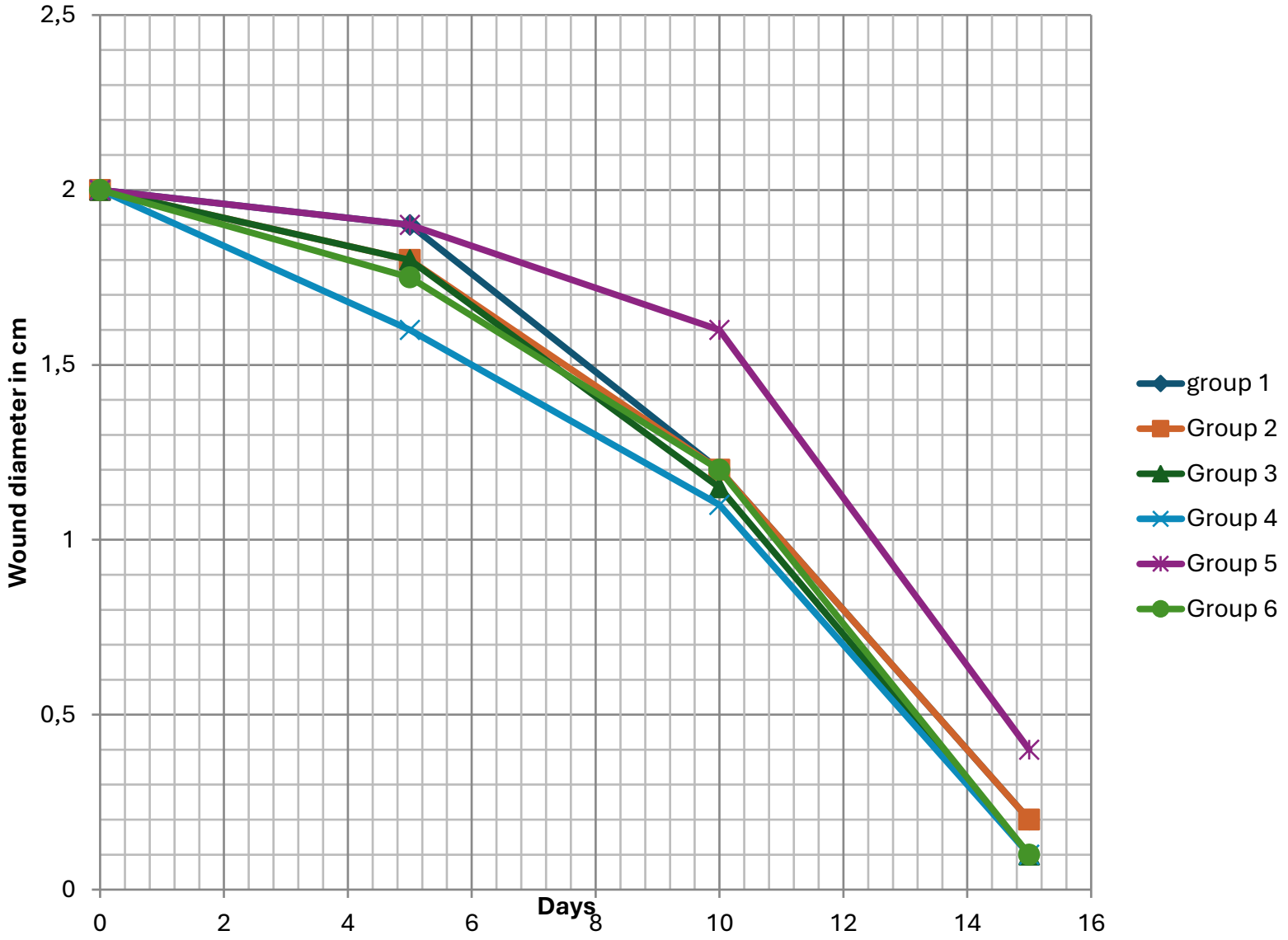

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Fig 2: Wound treated with 25% honey on 5th , 10th and 15th day.

Fig 3: Wound treated with 50% honey on 5th , 10th and 15th day.

Fig 4: Wound treated with 75% honey on 5th , 10th and 15th day.

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Fig 5: Wound treated with 100% honey on 5^{th} , 10^{th} and 15^{th} day.

Fig 6:Wound treated with neomycin ointment on 5^{th} , 10^{th} and 15^{th} day.

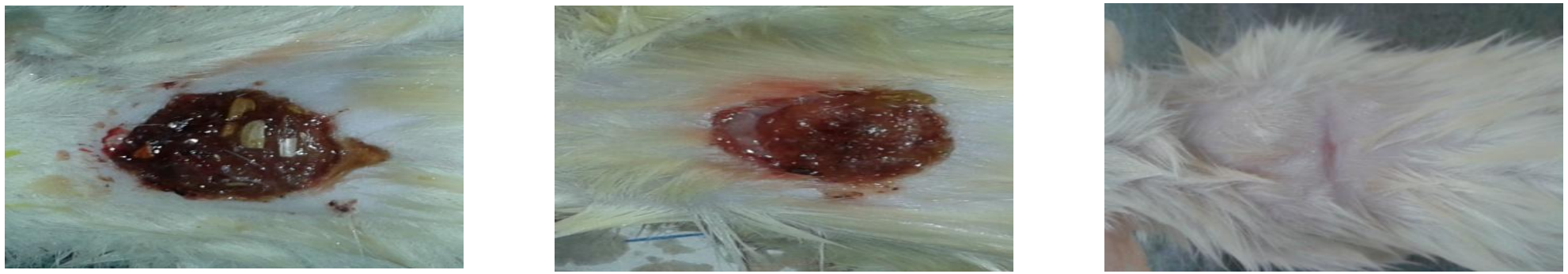
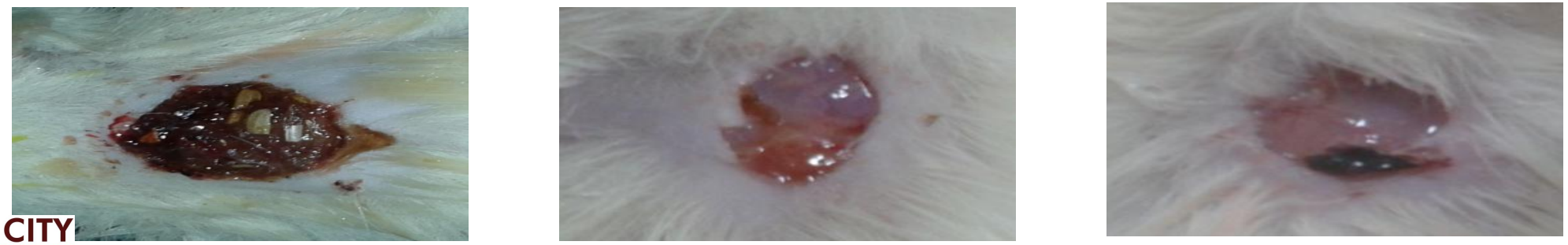


Fig 7:Wound treated with saline water on 5^{th} , 10^{th} and 15^{th} day.



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Antibacterial assay:

The honey samples were pasteurized according to the technique of Becker et al., 1996.

Erlenmeyer flasks (150 mL) with 50mL of nutrient broth medium were inoculated with the 10 ml of bacterial suspension and each concentration of honey namely 25%,50%,75% and 100% to be tested was added.

The specific growth rate (μ) values of yeast cultures were monitored by measuring optical density at 640 nm in an ultraviolet–visible spectrophotometer for every 30 minutes.

Results

Table 1: Specific growth rates of *E.coli* in honey containing media.

Honey samples	Specific growth rates (min^{-1}) at 640 nm			
	25%	**50%**	**75%**	**100%**
AC1	0.017	0.015	0.013	0.012
AC2	**0.019**	0.014	0.013	0.013
AP	0.018	0.018	0.013	0.012
AC4	0.017	0.016	0.016	0.013
AC5	0.021	0.021	**0.019**	0.017

- **Table 3: Specific growth rates of *B.subtilis* in honey containing media**

Samples	Specific growth rates (min^{-1}) at 640 nm			
	25%	**50%**	**75%**	**100%**
AC1	0.014	0.013	0.011	0.01
AC2	0.014	0.013	0.011	0.011
AP	0.018	0.017	0.017	0.013
AC4	0.019	0.018	0.017	0.017
AC5	**0.022**	0.021	0.019	0.015

BIOCHEMICAL ANALYSIS

Blood was collected from animals from each group on 5^{th},10^{th} and 15^{th} day and serum was separated to measure the level of albumin.

Serum albumin is a significant factor in wound healing, as it is a key protein involved in nutritional

status, inflammation, and providing amino acids necessary for tissue repair.

While a "normal" range for serum albumin in healthy adults is typically between **3.5 and 5.0 g/dL**,

studies suggest that maintaining levels within or above this range is generally desirable for optimal

wound healing.

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Groups	Day 5	Day 10	Day 15
Group 1	2.37g/dL	3 g/dL	3.81 g/dL
Group 2	2.32 g/Dl	3.05 g/dL	**4.1 g/dL**
Group 3	2.36 g/dL	2.99 g/dL	**4.34 g/dL**
Group 4	2.45 g/dL	3.18 g/dL	**4.87 g/dL**
Group 5	2.22 g/dL	2.63 g/dL	2.8 g/dL
Group 6	2.89 g/dL	2.91 g/dL	3.5 g/dL

Table 9: Serum albumin level

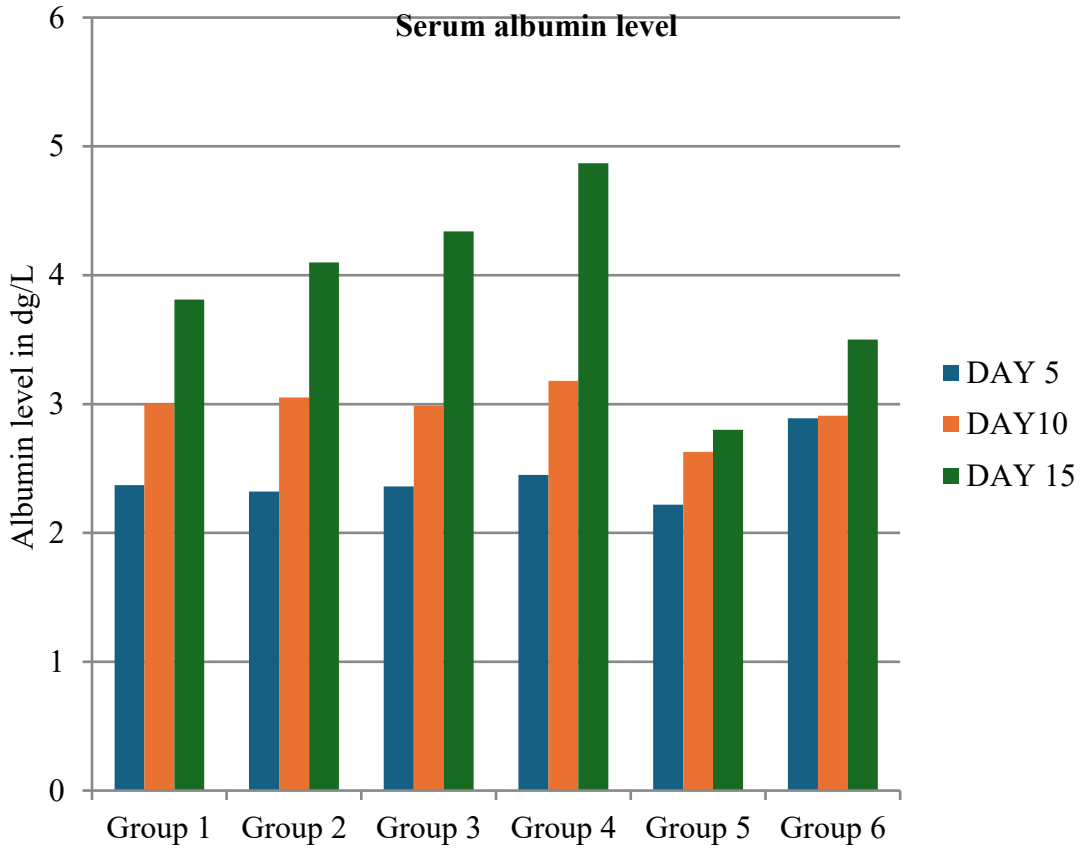

Fig 8: Serum albumin level

In the present study, early appearance of granulation tissue in group IV could be attributed to antimicrobial action of 100% honey that created a clean and healthy environment free from pus, toxins and microbes which is required for tissue regeneration.

In groups II and III, the early regeneration of granulation tissue could be attributed to the release of growth factors such as PDGF and TGF-β which in turn stimulate the migration and proliferation of fibroblast from the surrounding tissues and endothelial cells from injured blood vessels.

The rate of wound contraction was found to be superior in animals treated with the honey from day 3 onwards until day 10 when they reached the highest significant reduction as compared to that of low concentrations of honey and neomycin ointment.

The rapid wound closure observed in group IV could be attributed to the triterpenoid fraction present in the honey that stimulated the biosynthesis and maturation of collagen which caused wound contraction and quicker maturation and cross linking of collagen.

The triterpenoid compounds of the honey might have evoked the expression of genes involved in angiogenesis and the remodelling of the extra cellular matrix, as well as diverse growth factor genes.

The faster wound closure noticed in group IV animals could be due to the tissue proliferating property and the angiogenic property of honey causing increased vasculature around the wound area. An increase in the mRNA transcripts of transforming growth factor-β1 and fibronectin in treated wounds might have contributed to the faster healing observed.

In summary, honey showed significant wound healing activity in laboratory animals, as evidenced by gross appearance, graphical mapping, biochemical and histopathological studies.

The wound healing property of the polyherbal preparation was better than that of the widely used neomycin ointment and saline water.

The efficient healing action could be attributed to the increased collagen deposition, neo vasularization, epithelialisation, effective scavenging of reactive oxygen species, as well as the better alignment and maturation of cells involved in the reparative process at the wound site.

However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities. It is concluded that the honey had better wound healing activity than that of the widely used neomycin ointment.

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Many surgeons believe that nutritional state is the most important systemic factor affecting wound healing.

Honey treatment, especially in combined form, significantly improved the nutritional state of the treated animals as reflected by enhancement of serum albumin level.

In addition to sugars, which are important source of energy, honey contains amino acids, vitamin B's and C and minerals such as iron, copper and zinc.

These elements are cofactors for some of the enzymes involved in tissue repair such as proline hydroxylase, DNA-polymerase and Lysol oxidase.

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