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RESEARCH ARTICLE

COMPERATIVE STUDY OF NEEM, GARLIC, AND HENNA WITH ANTIBIOTIC NEBANOL® AGAINST ARTIFICIAL WOUND IN GUINEA PIGS.

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Abstract

Natural products are found to be more effective with least side effects as compared to commercial antibiotics so that reason alternated remedy are used for treatment of various infections. The aim of the present study was to evaluate the possible comparative efficacy of Neem (*Azadirachta indica*), Garlic (*Allium sativum*) and Henna (*Lawsonia alba*) with Antibiotic Nebanol (Neomycin Sulphate and Bacitracin Zinc) on artificially induced wound in 3cm length and 0.5 cm depth were made on the two thigh muscles an experimental model in Guinea pigs. The test animals were randomly chosen and divided into five groups having five Guinea pigs in each. Group-I, Group -II, Group -III received Neem leaf paste, Garlic paste, Henna paste and Group- IV received standard antibiotic Nebanol powder respectively. Group-V was kept as control and received vehicles only (distilled water containing 0.1% Tween-80). Neem leaf paste, Garlic paste, Henna paste, & Nebanol treatment reduced the wound within 12, 14, 17 & 13 days respectively. On the other hand, wound in control group was automatically reduced within 21 days. The present study showed that Neem was best effective among all & was found better than antibiotic Nebanol. It is concluded that among three preparations Neem may be used against skin wound because it is less expensive, easily available and having less side effect.

Introduction:-

The skin, as the body's largest organ consists of two layers, epidermis and dermis (Mescher, 2010). Human skin provides protection for the body from external environment, preventing dehydration, regulates body temperature, detects cutaneous sensation, and synthesizes vitamin D (Tortora and Derrickson, 2008). The skin barrier can be broken, such as from a wound (Torpy *et al*., 2005). There are many types wound which have been classified into two groups, open and closed wound (Chandler, 2011). Wound healing process is a biological process instigated by trauma and causes scar formation. Wound healing process occurs in few different stages such as coagulation, epithelisation, granulation, collegenation and remodelling of tissue. Wound healing is an active and multifaceted process in restoring cellular structures and tissue layers. The objective of wound management is to heal the wound in express time possible, with very nominal pain, discomposure and scarring in patient with wound. At the site of closure, a lithe and fine scar with high tensile strength is required (Bairy and Rao, 2001).

Plants have been an important source of medicine for thousands of years. As per World Health Organization estimates, up to 80 percent of people still depend on traditional remedies such as herbs for their medicines. Today, Ayurvedic, Hoemoeo and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Material Media (Narayana and Thammanna, 1987). Many plants have proved to possess

significant healing properties in different types of wounds. Using certain plants, possessing antiseptic, astringent, anti-inflammatory, antimicrobial property the rate of wound healing can be enhanced (Jaiswal *et al*., 2004). Such plant can increase the rate of tissue healing by providing different essential substances, required at various steps of wound healing. Plants being cheaper and safer than allopathic drugs, so treatment by natural ways may be beneficial in human and veterinary practice, especially in India where these are found in plenty (Wallis, 2004)

Azadirachta indica is a plant commonly known as Neem, belongs to the family Meliaceae (Chopra *et al*., 1956 & Nadkarni, 1954) used for tiredness, cough, fever, loss of appetite, worm infestation. It heals wound, vomiting, skin diseases, boils, jaundice, leprosy, skin & eye disorders, stomach ulcers, chicken pox, excessive thirst, diabetes, and insect poisons. It acts as antileprotic, antimalarial, anti-hemorrhoidal and anthelmintic agent etc (Ram and Mehrotra, 1984; Anonymous, 1962; Anonymous, 1996). Based on the above source of information, the present study aimed to evaluate the wound healing activity of *Azadirachta indica* (Neem) leaves. Biologically active principles isolated from different parts of the plant include: Azadirachtin, meliacin, gedunin, nimbidin, nimbolides, salanin, nimbin, valassin, meliacin forms the bitter principles of Neem oil, the seed also contain tignic acid responsible for the distinctive odour of the oil (Sharma *et al*., 2011). Neem kernels contain 30-50 % of oil mainly used by the soap, pesticide and contain many active ingredients which are together called triterpene or limnoids (Djenontin *et al*., 2012). The four best limnoids compounds are: Azadirachtin, Salannin, Meliantriol, and Nimbin. Limonoids contain insecticidal and pesticidal activity (Mondal *et al*., 2012).

Allium sativum L. is a member of the Alliaceae family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders. Most commonly used worldwide for flavorful cooking; much for the clinical literature on garlic has focused on its potential Antihypertensive (Ried *et al*., 2008), Wound Healing (Srimuzipo *et al*., 2009), Anticancer (Lau *et al*., 1990), Antiatherosclerosis and Hypolipidemic (Ashraf *et al*., 2005), Antimicrobial (Ankri *et al*., 1999), Antifungal (Davis *et al*., 1990), Immunomodulatory (Kyo *et al*., 2001), Antioxidant (Tsai *et al*., 2005), Antiinflammatory (Hodge *et al*., 2002), Antihelmentic activity (Worku *et al*., 2009). The most important chemical constituents reported from *Allium sativum* are the sulfur compounds (Bradley, 1992). It has been estimated that cysteine sulfoxides (e.g. alliin [Anonymous, 1985]) and the non-volatile γ-glutamylcysteine peptides make up more than 82% of the total sulfur content of garlic (Sendl *et al*., 1995). The thiosulfinates (e.g. allicin [Anonymous, 1997]), ajoenes (e.g. E-ajoene [Iwv, 1993], Z-ajoene [Jilid and Jakarta, 1995], vinyldithiins (e.g. 2-vinyl-(4H)-1, 3-dithiin [Anonymous, 1990], 3-vinyl-(4H)-1, 2-dithiin [Anonymous, 1953]), and sulfides (e.g. diallyl disulfide [Bradley, 1992], diallyl trisulfide [Youngken, 1950)]), however, are not naturally occurring compounds. Rather, they are degradation products from the naturally occurring cysteine sulfoxide, alliin (Anonymous, 1985). When the garlic bulb is crushed, minced, or otherwise processed, alliin is released from compartments and interacts with the enzyme alliinase in adjacent vacuoles. Hydrolysis and immediate condensation of the reactive intermediate (allylsulfenic acid) forms allicin (Anonymous, 1997). One milligram of alliin is considered to be equivalent to 0.45mg of allicin (Block, 1985). Allicin itself is an unstable product and will undergo additional reactions to form other derivatives (e.g. products [Iwv, 1993]), depending on environmental and processing conditions (Block, 1985). Extraction of garlic cloves with ethanol at 0°C gave alliin (Anonymous, 1985); extraction with ethanol and water at 25°C led to allicin (Anonymous, 1997) and no alliin; and steam distillation (100°C) converted the alliin totally to diallyl sulfides (Sendl *et al*., 1995). Sulfur chemical profiles of Bulbus Allium sativum products reflected the processing procedure: bulb, mainly alliin, allicin; dry powder, mainly alliin, allicin; volatile oil, almost entirely diallyl sulfide, diallyl disulfide, diallyl trisulfide, and diallyl tetrasulfide; oil macerate, mainly 2-vinyl-[4H]-1,3-dithiin, 3vinyl-[4H]-1,3-dithiin, E-ajoene, and Z-ajoene (Lawson *et al*., 1991). The content of alliin was also affected by processing treatment: whole garlic cloves (fresh) contained 0.25–1.15% alliin, while material carefully dried under mild conditions contained 0.7–1.7% alliin (Lawson *et al*., 1991). Gamma-glutamylcysteine peptides are not acted on by alliinase. On prolonged storage or during germination, these peptides are acted on by γ-glutamyl transpeptidase to form thiosulfinates (Sendl *et al*., 1995).

Lowsonia alba commonly known as Henna or Mehedi and abundantly available in tropical and subtropical areas (Lavhate and Mishra, 2007). Henna has been used cosmetically and medicinally for over 9,000 years. Traditionally in Bangladesh, mehedi is applied to hands and feet. Henna symbolizes fertility. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent (Chetty, 2008; Chopra *et al*., 1956; Reddy, 1988). It also have Antidiabetic (Arayne *et al*., 2007), Immunomodulatory (Dikshit *et al*., 2000), Hepatoprotective (Hemalatha *et al*., 2004), Antioxidant (Dasgupta *et al*., 2003), Antibacterial (Ali *et al*., 2001), Antifungal (Singh *et al*., 1989), Antiviral (Khan *et al*., 1991), Antiparasitic (Okpekon *et al*., 2004),

Antidermatophytic (Natarajan *et al*., 2000), Tuberculostatic (Sharma, 1990), Antifertility (Munshi *et al*., 1977), Analgesic. (Bagi *et al*., 1988), and Anti-inflammatory (Gupta *et al*.,1993) properties. The principal coloring matter of henna is lawsone, 2hydroxy-1:4 napthaquinone besides lawsone other constituents present are gallic acid, glucose, mannitol, fats, resin (2 %), mucilage and traces of an alkaloid. Leaves yield hennatannic acid and an olive oil green resin, soluble in ether and alcohol.

From the results attained in present investigation, it is feasible to conclude that the Neem (*Azadirachta indica*), Garlic (*Allium sativum*) and Henna (*Lawsonia alba*) has significant wound healing activity at the doses tested on excision wound model in animal study. However, further studies should be done to prove the potential of Neem, Garlic and Henna in wound healing using other wound models such as incision and dead space wound.

Materials and methods:-

Plant materials:-

Neem, Garlic and Henna are available in Chittagong, Cox's Bazar, Barguna, Jhalukati, Rajshahi, Pabna, Jessore, Kustia, Faridpur, Dhaka, and all over in Bangladesh. For our study the sample was were collected from the Gazipur near Dhaka and identified by the experts of Bangladesh National Herbarium, Dhaka.

Preparation of Medicinal Paste:-

The Neem and Henna are carefully cut with the help of a scissor and separated from other parts. The papery surfaces of Garlic have been separated first. The raw Neem leaf, Garlic and Henna were cleansed with fresh water. Then the raw Neem, Galic and Heena were crushed separately thoroughly with the help of pestle and mortar. The raw Neem, Galic and Heena were then mixed with distilled water and grinded them as 60% solution. The pastes were then preserved in the separate plastic container.

Drug:-

Nebanol (Neomycin Sulphate and Bacitracin Zinc), (Square Pharmaceuticals Limited, Bangladesh)

Animals:-

Male and female Guinea Pigs of either sex (450-600 gm body weight), Age: 6-8 month bred in the animal house of the Department of Pharmacology, Bangladesh Agricultural University, were collected from the animal resources branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B).The animals were housed under standard laboratory conditions (relative humidity 55-60%, r.t 23±2^{o}C and 12 hour light: dark cycle).The animals were fed with standard diet and water *ad libitum.* The institutional animal ethical committee approved the study protocol.

Wounds Preparation:-

Before surgery, all the animals were subjected to a thorough clinical examination to ensure that they were in good physical condition and apparently free from infections or parasitic diseases. The operation sites both thighs were clipped, washed with soap and water, shaved and prepared with Iosan (Ciba Geigy, Switzerland Ltd). The sites were desensitized by local infiltration with 2% Jasocaine (Lignocaine hydrochloride, Jayson, Bangladesh Limited). Two incised wounds of 3 cm length and 0.5 cm depth were then made on either side of horizontal column following standard surgical procedure.

Study Design:-

After making wounds, the Guinea pigs were divided into 5 equal groups. Each comprising five animals and marked as group – I, II, III, IV and group V. **Group- I:** Paste of Neem leaf was applied to the 10 wounds made in 5 animals for twelve days. The paste was used twice daily after washing the wounds by distilled water with the help of sterile cotton. **Group- II:** Paste of Garlic was applied to the 10 wounds made in 5 animals for fourteen days. **Group- III:** Paste of Henna leaves was applied to the 10 wounds made in 5 animals for seventeen days. **Group- IV:** Antibiotic (Nebanol powder) was applied to the 10 wounds made in 5 animals for thirteen days. **Group- V:** No medicine was applied to the 10 wounds made in 5 animals.

Table 1: Used medicinal plants and experimental drugs as study designed

Group	Medicinal Plants Name	Part Used	Number of Animal	Daily Application
Group- I	Neem (*Azadirachta indica*)	Leaf Paste	5	Twice
Group- II	Garlic (*Allium sativum*)	Whole Parts Paste	5	Twice
Group- III	Henna (*Lawsonia alba*)	Leaf Paste	5	Twice
Group- IV	Nebanol (Neomycin Sulphate and Bacitracin Zinc)	Powder	5	Twice
Group- V	Control	-	5	-

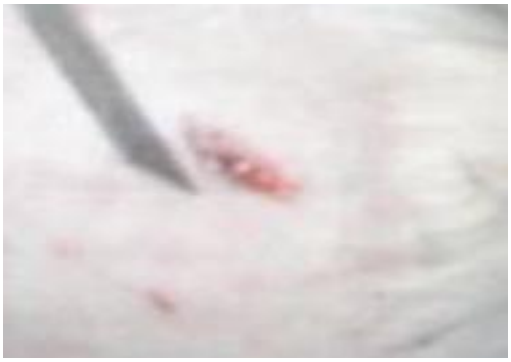

Fig-1: Artificially induced wound

Fig-2: Healing Process of Garlic

Fig-3: Healing Process of Heena

Fig-4: Healing process of Neem

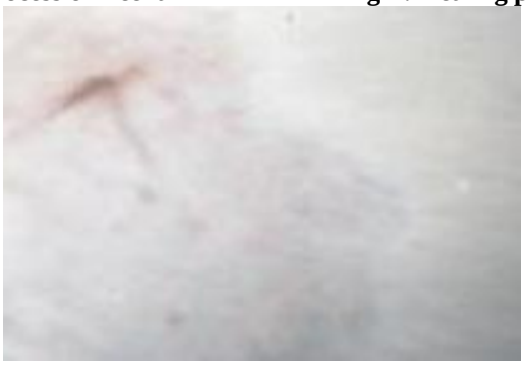

Fig-5: Healing process of Nebanol

Clinical Parameters:-

Healing of wound and Hematological Parameter:-

A drop of blood was collected from the ear vain of each experimental animal before wounding and on 3rd, 10th, and 14th days of wounding. The blood was analyzed for Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Haemoglobin (Hb) & Differential Leukocyte Counts (DLC) (Coffin, 1955).

Results:-

The comparative efficacy of three medicinal plants Neem, Garlic, Heena and locally available drug Nebanol against artificially induced wound have been examined in Guinea pigs. Complete healing time of the artificially induced wound in various treatment groups vary with the effectiveness of the treated materials which are shown in table 2 and 3. All pastes seemed to be effective for wound healing. The comparative efficacy of three medicinal plants and locally available drug nebanol against wounds are shown in Table-2. The paste of Neem showed the best results where healing was complete in 12 days.

Table 2: Healing time of the wounds in various treatment groups

Group	Name of the Medicine	Completed Healing Time
I	Neem	12 days
II	Garlic	14 days
III	Heena	17 days
IV	Nebanol	13 days
V	Control	21 days

Neem pastes were more effective than those of Garlic and Heena. Antibiotic Nebanol took 13 days for healing. The garlic paste and Heena showed the different results where healing were completed in 14 and 17 days respectively. The control group took more time than Neem, Garlic paste, Heena and antibiotic and it was 21 days. The characteristic clinical changes at different stages of wound healing with various preparations are shown in Table 3. Moderate exudation occurred on the first day of wound healing. The wounds remained reddish from 3rd to 9th day in all treatments groups. The redness in Neem paste groups however was more prominent.

Table 3: Characteristic clinical features at the different stages of wound healing

Day	Changes	Neem Paste	Garlic Paste	Heena Paste	Nebanol	Control
1st	Length of wound (cm)	3	3	3	3	3
	Exudation	+	+	+	+	+
	Reddening	-	-	-	-	-
	Cavity filling (%)	-	-	-	-	-
3rd	Length of wound (cm)	2.57±0.033	2.68±0.027	2.81±0.065	2.72±0.036	2.79±0.022
	Exudation	-	-	-	-	-
	Reddening	+	+	+	+	+
	Cavity filling (%)	10	-	-	-	-
5th	Length of wound (cm)	2.11±0.023	2.32±0.026	2.49±0.042	2.21±0.037	2.68±0.025
	Exudation	-	-	-	-	-
	Reddening	++	++	++	++	++
	Cavity filling (%)	40	20		30	
7th	Length of wound (cm)	1.58±0.043	1.92±0.029	2.12±0.047	1.71±0.028	2.4±0.023
	Exudation	-	-	-	-	-
	Reddening	++	+++	++	+++	++
	Cavity filling (%)	60	40	20	50	10
9th	Length of wound (cm)	0.69±0.014	1.41±0.019	1.79±0.031	1.12±0.022	2±0.031
	Exudation	-	-	-	-	-
	Reddening	++	++	++	++	++
	Cavity filling (%)	80	60	40	70	20
12th	Length of wound (cm)	Healing	0.79 ±0.027	1.42±0.044	0.4±0.024	1.82±0.017
	Exudation	complete on	-	-	-	-
	Reddening	12th day,100%	+	+	+	+
	Cicatrisation		+++	+	+	-
	Pigmentation		+	+++	+	-
	Cavity filling %		80		90	
13th	Length of wound (cm)	-	0.42±0.018	1.11±0.023	Healing	1.61±0.011
	Exudation	-	-	-	complete on	-
	Reddening	-	-	-	13th	-

					day,100%	
	Cicatrisation	-	+	+		+
	Pigmentation	-	+	++		+
	Cavity filling %	-	90	60		40
14th	Length of wound (cm)	-	Healing	0.63±0.018	-	1.43±0.028
	Exudation	-	complete on	-	-	-
	Reddening	-	14th day,100%	-	-	-
	Cicatrisation	-		+	-	+
	Pigmentation	-		+	-	++
	Cavity filling %	-		80	-	60
17th	Length of wound (cm)	-	-	Healing	-	0.61±0.033
	Cicatrisation	-	-	complete on	-	+
	Pigmentation	-	-	17th day,100%	-	++
	Cavity filling %	-	-	-	-	80
21th	Length of wound (cm)	-	-	-	-	Healing
	Cicatrisation	-	-	-	-	complete on
	Pigmentation	-	-	-	-	21th day,100%
	Cavity filling %	-	-	-	-	-

In case of garlic paste, Heena paste and nebanol groups, redness were observed from the 3rd to 12th day. But on 9th day, it was more prominent. In Neem groups, 30-35% of the wound cavity was filled up at the same time of the treatment. At the 10th day of wounding a strong scab was observed above the wound and 85-95% cavity was filled up when the paste of Neem was used. At the same time the filling of the cavity in other groups occurred on the 12th to 15th day.

The complete healing of the wound has been occurred within 12th day by using Neem paste while for other groups i.e. garlic, henna and patent drug nebanol complete filling of the wound occurred within 13th to 17th day. In control group complete cavity filling was observed on the 21st day.

TEC, TLC, and Hb concentration:-

The effects of external wound on total leukocytes counts (TLC), total erythrocytes count (TEC), and hemoglobin concentrations are demonstrated in respectively. The total erythrocyte count TEC before producing wound in the animals of group I, II, III, IV and V were 17.81± 1.3, 17.23± 1.4, 17.36± 1.5, 17.58± 0.9 and 17.21± 1.3 millions/cu mm respectively. These mean values decrease to 14.11± 1.2, 14.54± 0.9, 13.41± 0.8, 13.5± 1.2 and 14.32± 0.9 millions/cu mm on 3rd days in every group and continued to decreased 12.56± 1.4, 13.58± 2.1, 12.77± 1.7, 12.64± 1.8 and 13.63± 2.4 million/cu mm until 10th days. However, these decreases were not statistically significant.

Table 4: effect of three selected medicinal plants and antibiotic on tec, tlc and hemoglobin concentration of external wound in guineapigs

Reading	**TEC (million/cu mm)**					**TLC (thousands/ cu mm)**					**Hemoglobin (%)**				
Stage	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Before wound	17.81 ± 1.3	17.23 ± 1.4	17.36 ± 1.5	17.58 ± 0.9	17.21 ± 1.3	14.2 ±1.1	14.1 ±1.3	14.4 ±0.9	14.3 ±0.8	14.5 ±1.1	7.76 ±0.3	6.98 ±1.1	6.63 ±1.3	7.52 ±0.9	7.22 ±0.7
3 days after wound	14.11 ±1.2	14.54 ±0.9	13.41 ±0.8	13.5 ±1.2	14.32 ±0.9	23** ±0.6	15.4 ±0.5	22.3** ±0.6	23** ±0.8	22.2** ±0.4	7.53 ±0.5	6.77 ±0.7	6.59 ±0.6	7.32 ±1.3	6.86 ±1.2
10 days after wound	12.56 ± 1.4	13.58 ± 2.1	12.77 ± 1.7	12.64 ± 1.8	13.63 ± 2.4	21** ±1.2	15.5 ±1.7	23.4** ±0.8	22** ±1.3	21.9** ±0.6	6.88 ±0.6	6.46 ±1.3	6.46 ±1.1	7.11 ±0.4	6.53 ±0.7
14 days after wound	14.85 ±1.6	14.63 ±1.6	15.71 ±1.7	15.12 ±1.8	15.15 ±1.9	14.8 ±0.8	15.1 ±0.9	16.3 ±1.2	15.4 ±1.5	16.1 ±1.8	6.51 ±1.4	6.67 ±0.9	6.67 ±0.5	6.82 ±0.7	6.23 ±0.4

I= Neem Group, II= Garlic Group, III= Heena Group, IV= Nebanol Group, V= Control Group
**p<0.001 and *p<0.01 vs. control, Values are expressed as mean ± SEM (n = 5)
On 14th days these valued increase to 14.85± 1.6, 14.63± 1.6, 15.71± 1.7, 15.12± 1.8, 15.15± 1.9 million/cu mm in group I, II, III, IV and V respectively. Among the five different groups the highest TEC count was found in group III at 14th day's 15.71± 1.7 million/cu mm respectively. However, the differences of total erythrocyte count TEC, among the five different groups were not statistically significant, Table 4.

The total leukocyte count TLC before producing wound in the animals groups I, II, III, IV, V were 14.2± 1.1, 14.1± 1.3, 14.4± 0.9, 14.3± 0.8, 14.5± 1.1 thousands/cu mm respectively. These mean values increased significantly p<0.001 in group I and III to 23± 0.6, 22.3± 0.6 and 21± 1.2, 23.4± 0.8 thousands/cu mm on 3rd days and 10th days after producing wound. In group II, IV and V total leukocyte count (TLC) also increased respectively. However, these changes were not statistically significant p>0.5, Table 4.

The mean hemoglobin Hb concentration in group I, II, III, IV and V were 7.76± 0.3, 6.98± 1.1, 6.63±1.3, 7.52 ± 0.9 and 7.22 ± 0.7 gm%. The hemoglobin concentration in different groups did not vary significantly on different days of wound, Table 4.

Differential leukocyte count (DLC):-

The effect of experimental wound on differential count and the individual data are presented in the following Table-5. Among the leukocyte, the noticeable changes were observed in lymphocytes and neutrophils. The mean values of lymphocytes before producing wound in groups I, II, III, IV and V were 67.8± 1.5, 66.21± 3.4, 72.36± 1.5, 67.58± 2.9 and 68.9± 3.3 percent respectively. These values increase significantly P<.001 to 84± 1.3 and 85.1± 0.8, 83± 1.4, 82.7±2.7 percent on 3rd and 10th day in group I and III respectively. In group II, IV and V after producing wound lymphocytes also increased to 74.54± 2.3, 83.5± 2.2, and 78.32± 3.9 in 3rd day. 10th day and 14th day also increased respectively. However these changes were not statistically significant.

Table 5: effect of three selected medicinal plant and antibiotic on differential count of leukocyte (dlc) of external wound in guinea pigs

Reading	**Lymphocytes (%)**					**Neutrophils (%)**					**Eosinophils (%)**				
Stage	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Before wound	67.8 ± 1.5	66.21 ± 3.4	72.36 ± 1.5	67.58 ± 2.9	68.9 ± 3.3	29.2 ±2.1	28.1 ±1.3	24.4 ±1.9	26.3 ±3.8	28.5 ±1.9	3.76 ±0.3	3.98 ±1.1	2.63 ±1.3	4.52 ±0.9	3.82 ±0.9
3 days after wound	84** ±1.3	74.54 ±2.3	85.1** ±0.8	83.5 ±2.2	78.32 ±3.9	24.8 ±1.6	26.4 ±3.9	22.8 ±0.9	25.9 ±2.8	26.1 ±2.4	3.53 ±0.5	3.77 ±1.7	4.59 ±0.6	4.32 ±1.3	3.86 ±1.2
10 days after wound	83** ± 1.4	73.68 ± 2.1	82.7** ± 2.7	79.6 ± 2.8	78.6 ± 2.9	24.4 ±1.2	26.5 ±2.7	23.4 ±1.8	25.6 ±2.3	24.9 ±2.6	3.88 ±2.6	2.46 ±1.3	5.48 ±1.1	4.71 ±0.7	4.53 ±1.7
14 days after wound	64.8 ±1.5	79.63 ±1.7	65.71 ±1.3	68.12 ±1.6	74.8 ±2.8	28.8 ±2.8	28.1 ±1.9	26.3 ±1.4	26.4 ±1.5	28.3 ±3.8	3.51 ±1.4	2.67 ±1.9	5.62 ±1.5	4.12 ±1.7	4.26 ±1.4

I= Neem Group, II= Garlic Group, III= Heena Group, IV= Nebanol Group, V= Control Group
**p<0.001 and *p<0.01 vs. control, Values are expressed as mean ± SEM (n = 5)
The mean values of neutrophils before producing wound in group I, II, III, IV and V were 29.2± 2.1, 28.1± 1.3, 24.4± 1.9, 26.3± 3.8, and 28.5± 1.9 percent respectively. After producing wound these values on 3rd and 10th days started to decrease gradually in every group and the lowest was 22.8± 0.9 percent in group III on 3rd days. On 14th days these value again increase and highest value was in group I is 29.2± 2.1 percent. However, these changes were not statistically significant among the three different groups.

The changes in eosinophil in various groups at different days were inconsistent and statistically insignificant

Discussion:-

Wound healing involves a highly dynamic integrated series of cellular physiological and biochemical processes that occurs in living organisms (Srinivas *et al*., 2008; Mukherjee *et al*., 2002). The majority of world population relies on traditional medicine for their health care (Zhang, 1996). This is also the case in the treatment of wounds. Many research proposed that wound healing can be improved by herbal drugs having antiseptic, antibacterial, antioxidant and anti-inflammatory properties (Somashekar *et al*., 2006; Sunil *et al*., 2008). In the present study experimental skin wounds were produced artificially in the Guinea pigs and the sequence of the events during wound healing were studied. The results showed that in the hematological study of different counts of leukocytes, significant leukocytosis was observed in medicinal plants treated group, antibiotic group and control groups. In Nebanol powder treated group insignificant leukocytosis was observed which might be due to the effect of antibiotics against wounds. The possible explanation of this effect may be that external wounds causes leukocytosis but this may be inhibited due to antibiotic therapy. Leukocytosis is the indication of inflammatory process, which is essential for healing mechanism. Increased level of leukocytosis also produces various enzymes, which destroy the host cells and may further perpetuate the condition and delay wound healing (Williama and Deniel, 1986). The wound inflicted in the present experiment reminded exposed. The relatively larger healing time may be associated with the wound exposure, resulting the increase dehydration of both the wound edges and the base and so lead to greater local tissue death and enlarged scab formation and a slower rate of epithelization. Epithelixation is accompanied by the laying down scar tissue, which is composed of collagen tissue and is devoid of nervous tissue, sweat glands, sebaceous gland and hair follicles (Walton and Neal, 1972). The wounds remained reddish from 3rd to 9th day. This indicated the formation of granulation tissue, which restores the wound gap. The wound cavity was filled up in between 9 and 21 days in various treatment groups and these were similar with the findings of Pandey and Ghani 1986 (Pandey *et al*., 1986). During wound healing species variation showed marked difference die to external environmental factors and in addition they show an individual idiosyncrasy. Poor or high level of nutrition and in specific protein deficiency may cause delayed healing. The rise of skin temperature enhances epithelial regeneration and in regeneration of fibroblast elements in the sub-epidermal tissues (Silver, 1973). In the present study, Neem paste was found best 12 days in wound healing than two other plants paste and a patent drug Nebanol. Neem, Garlic, Heena and its paste can industrially be manufactured. During this research all pests and powder were preserved in the refrigerator and was used in various time. However, identification of the active principle of the plants was not done in this study because of insufficient laboratory facilities. So these works may be performed in future.

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