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WOUND HEALING AND ANTIOXIDANT POTENTIAL OF CHLOROFORM EXTRACT OF SARACA *ASOCA* (ROXB.)

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ABSTRACT: The chloroform extracts of *S. asoca* bark were studied for the wound healing potential. The wound healing activity of *S. asoca* bark by excision, incision and dead space wound models in albino Wistar rats and antioxidant profile of this bark extract were also studied as there is an increase in the levels of free radicals during the damage of tissue. The parameters like wound contraction, period of epithelialisation, tensile strength and hydroxyproline content were evaluated in three wound models, namely; excision, incision and dead space wound models. Besides, their influences on oxidative stress in three wound models were also estimated. The results obtained were compared with standard drug nitrofurazone and control in terms of wound contraction, period of epithelialisation, tensile strength and hydroxyproline content. The chloroform extract of S. asoca bark showed significant results in all models when compared to standard and control. A subservient study made on the levels of superoxide-dismutase, catalase, glutathione, vitamin C and lipid peroxidation are recorded and a significant increase in the levels of these antioxidant enzymes and decrease in the levels of lipid peroxidation was observed. The histopathological studies have shown the well-formed dermis with minimal inflammatory cells. Increased wound contraction, collagen migration, granulation tissue formation is attributed to the phytochemical constituents present in this plant extract. Increased levels of antioxidants favour the regeneration of wounds by quenching free radicals. Phytochemical constituents present in *S. asoca* may be responsible for the wound healing activity.

INTRODUCTION: Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed the functional status of the skin [1].

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Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferative phase, and finally the remodelling phase which ultimately determines the strength and appearance of the healed tissue [2].

The basic principle of optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion and oxygenation [3]. Antioxidant activity was determined since the

release of oxygen radicals kills invading foreign organisms and clears the wound of fibrin matrix, thus enhancing the healing process, whereas fibroblasts play a crucial role in wound healing by initiating the proliferative phase of repair . More than 80% of the world's population still depends upon traditional medicines for various skin diseases [4].

Various plant species have served as a source of medicine for people all over the world. *S. asoca (*family: Caesalpinaceae) is a small evergreen tree 7-10 cm high. It occurs up to the altitude 750 meters. Bark is blackish brown in colour and rough due to warty protuberances and transversely arranged lenticles. It is of astringent taste and odourless character. *S. asoca* is one of the universal plant having medicinal activities.

Ashoka is ancient and reliable source of medicine. It is used in the treatment of uterine bleeding, dysmenorrhoea, depression in women, uterine fibroids, uterine sedative, leucorrhoea, piles, ulcers, astringent. Different extracts of *S. asoca* bark were screened against the enteric pathogen isolates, namely, *Escherichia coli*, *Shigella sonnei* and *Salmonella enteritis*.

All the extracts other than aqueous extract showed antimicrobial activity with the methanol extract having the highest percentage of activity [5]. The methanolic extract of *S. asoca* bark was also evaluated for analgesic and anti-inflammatory activity and it is reported to show significant activity [6].

As global scenario is now changing towards the use of nontoxic plant products having traditional medicinal uses, *S. asoca* is evaluated in the hastening of wound healing by promoting maturation of fibroblasts and in the management of hypertropic scars. The removal of reactive oxygen species that rises during the inflammatory phase of wound healing is essential to hasten the wound healing process.

Therefore, the estimation of antioxidants like vitamin c, glutathione, superoxide dismutase, catalase, lipid peroxidation is also relevant because these antioxidants hasten the process of wound healing by destroying the free radicals.

MATERIALS AND METHODS: Inbred house Albino and Wistar rats of either sex were used in the study. The range of the weight of the animals is between 200-250g. They were housed individually in standardized environmental conditions. All the animals were provided with food *ad libitum*.

Chemicals: Chloroform, O-dianisidine, hydrogen peroxide, riboflavin, 5% & 6% Trichloro acetic acid, Dinitrophenylhydrazine, Thiobarbituric acid were purchased from Sigma chemicals. Other chemicals and reagents used are of analytical grade.

Plant material and extraction procedure: Bark of *S. asoca* was collected from the village of Vadlamudi, Guntur district, AP India. It was identified by Dr. M.Raghuram (Department of Botany & Microbiology) Acharya Nagarjuna University, Guntur, Andhra Pradesh. They were shade dried and coarsely powdered. The powdered material (100g) was placed in soxhlet extractor and it is extracted with chloroform. The extract is evaporated and the dried residue (15g) i.e; drug is collected.

Phytochemical screening: Various chemical tests were performed to identify the phytochemical constituents present in the chloroform extract of *S. asoca* [7, 8].

Acute dermal toxicity: The acute dermal toxicity was carried out in adult albino rats by "fix dose" method of OECD (Organisation for Economic Co-operation and Development) (Guideline No.434). The chloroform extract of *S. asoca* was applied topically at dose level 2000mg/kg and it showed no signs of erythema.

Drug formulations: The chloroform extract of *S. asoca* was formulated as 5%w/w and 10%w/w ointments respectively. These ointments were prepared by incorporating 5g and 10g of drug respectively into 100g of simple ointment base. The ointment base consists of glycol stearate, 1, 2-propylene glycol, liquid paraffin (3:6:1). This was achieved by melting together with glycol stearate, 1, 2 propylene glycol, and liquid paraffin on a hot plate/stirrer (at 45 ◦C). The chloroform extract of *S. asoca* was added to this molten base while stirring.

The entire mixture was stirred while cooling until a smooth ointment was obtained. The prepared ointments were stored at room temperature. The standard drug used for wound healing activity was Nitrofurazone 0.2%w/w [9].

Animals: The animals were housed under controlled conditions (12 h light–dark cycle, 22–28 ◦C and 60–70% air humidity), fed with normal mice chow and *water ad libitum*. All animals were allowed to acclimate for at least 7 days prior to the first treatment. The rats were anesthetized before and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using light ether anesthesia. All animal experiments followed the guidelines for the care and use of animals established by Vignan Pharmacy College, Vadlamudi, and was approved by the Ethics Committee of Vignan Pharmacy College (7/IAEC/VPC/Pharma/RES/2011-12).

Experimental Protocol:

For each model; 4 groups were used. Each group consists of 6 animals.

Group 1, 2, 3, 4 – Served as control, standard, treatment groups receiving 5% & treatment groups receiving 10% ointment respectively for Incision wound model.

Group 5,6,7,8 – Served as control, standard, treatment groups receiving 5% & treatment groups receiving 10% ointment respectively for Excision wound model.

Group 9,10,11,12 – Served as control, standard, treatment groups receiving 5% & treatment groups receiving 10% ointment respectively for Dead space wound model.

Treatment Schedule: Chloroform extracts of *S. asoca* were mixed with ointment base and applied once a day to wounds of experimental animals until they were cured.

Wound Models:

Incision Wound Model: Two, 6 cms long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat [10]. Wounds were closed with interrupted sutures, 1 cm apart. The sutures were removed on the seventh day. Wound-breaking strength was measured in anesthetized rats on the tenth day after wounding.

Excision Wound Model: A rectangular skin piece of full thickness (approximately 500 mm^2) was removed from a predetermined dorsal area [11]. The wounds were traced on 1mm^2 graph paper on the day of wounding and subsequently on alternate days until healing was complete. The wound healing observations were shown in the **figure 3**.

Changes in the wound area were calculated, giving an indication of the rate of wound contraction. The number of days required for falling of the eschar without any residual raw wound was determined as the period of epithelisation.

The degree of wound healing was calculated using the formula:

Percentage closure = $1-A_d/A_0 \times 100$

Where A_d = wound area on corresponding days, A_0 = wound area on zero day

Histopathological examination: A specimen sample of skin tissues of each group of rats were taken out from the healed wounds of the animals in the excision wound model for histopathological examinations. The thin sections of the tissues were stained with Eosin I bluish solution and observed for the histological changes under microscope [12] and shown in the **figures 1 and 2**.

Dead-Space Wound Model: Dead space wounds were created through a small tranverse incision made in lumbar region [13]. A polypropylene tube (0.5 cm × 2.5 cm) was implanted subcutaneously beneath the dorsal paravertebral lumbar skin. The day of the wound creation was considered as day zero. Granuloma tissue formed on the implanted tube was dissected out carefully on day 10. The granulation tissue was dried in an oven at 60°C for 24hr and the dry weight was noted. The acid hydrolysate of the dry tissue was used for the estimation of hydroxyproline content in the tissue.

Biochemical estimations: On the 2nd day of wound induction and on complete healing blood was collected from retro orbital plexus and was estimated for the antioxidant activity [14].

Estimation of Superoxide Dismutase: To 3ml of packed blood cells equal volume of cold deionized water was added for the lysis of packed blood cells. Haemoglobin was then precipitated by the addition of chloroform and ethanol (1.5:1). This was then centrifuged for 15minutes at 3000rpm. To 100µl of supernatant 0.88ml of riboflavin and 60 µl of O-dianisidine was added and optical density was measured at 460nm [15].

Estimation of Catalase: To 0.1ml of serum 2.5ml of phosphate buffer was added and incubated for 30minutes. Later 650µl of hydrogen peroxide was added and measured at 240nm [16].

Estimation of Reduced Glutathione: To 0.5ml of citrated blood, 0.5ml of 5% trichloroacetic acid was added and centrifuged. To the supernatant1ml of sodium phosphate buffer and 0.5ml of DTNB reagent were added. The absorbance was measured at 412nm [17].

Estimation of Vitamin C: To 0.5ml of plasma, 6%TCA was added and centrifuged. 0.5ml of DNPH was added to supernatant and read at 530nm [18].

Estimation of Lipid Peroxidation: 0.1ml of plasma was treated with 2ml of TBA, HCL, TCA and placed in water bath for 15min, cooled centrifuged and the supernatant was measured at 535nm [19].

Statistical Analysis: All values were expressed as Mean ± SEM. The data was analysed using analysis of variance followed by student T-test for reporting the *p*-value and significance with respect to the other groups.

RESULTS: Preliminary phytochemical screening of the plant showed the presence of carbohydrates, steroids, alkaloids, cardiac glycosides, anthraquinone glycosides. In incision wound model, a significant increase was observed in the skin tensile strength of 10% extract treated group when compared to control and standard (**Table 1**).

In studies using excision wound model the area of the wound was measured on 2, 4, 6, 8 and 10 days of post-surgery in all groups. The control group treated with simple ointment has shown little contraction compared with 10% treated group. A very rapid closure of the wound was observed on day 8 and 10 of post-surgery ($p<0.0001$) (**Table 2**).

The total wound closure was observed by day 10 of post wounding in 10% treated group and it was 20 days in the control group. Figure 2 represents the 10% treated group showing minimal inflammation with well-formed epidermis when compared to figure 1 as they contains irregularly arranged fibroblasts and macrophages. Hydroxyproline is not directly coded by DNA; however, Proline is hydroxylated to form hydroxyproline after protein synthesis. Hydroxyproline is a major component of the protein collagen.

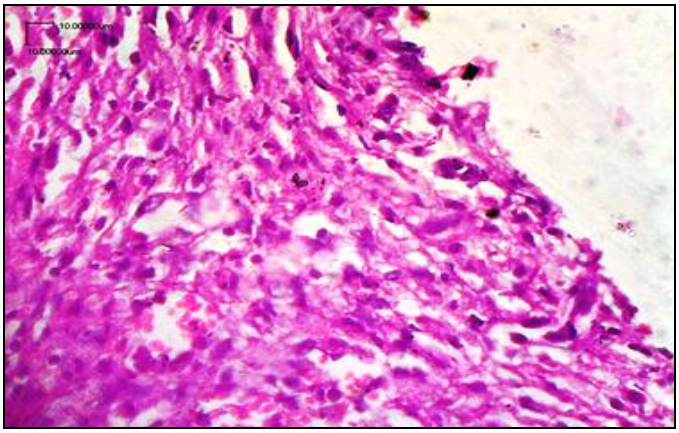

FIGURE 1: CONTROL (H&E 400×) SHOWING WELL-FORMED BUT THICK GRANULAR CELL LAYER, THE UNDERLYING DERMIS CONTAINS DEPOSITED COLLAGEN FIBERS AND IRREGULARLY ARRANGED FIBROBLASTS AND MACROPHAGES

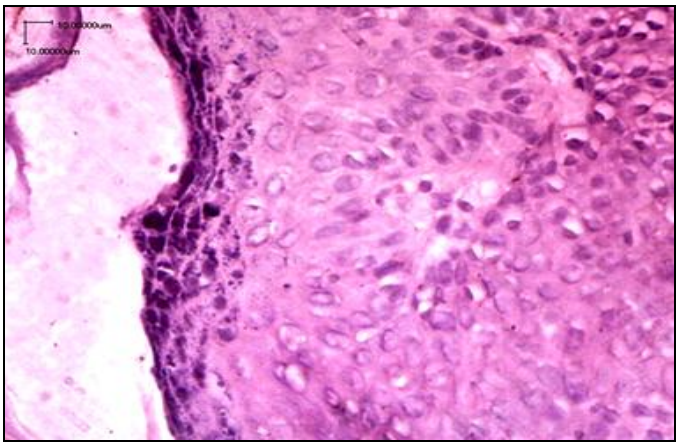

FIGURE 2: ANIMALS TREATED WITH *S. ASOCA* (H&E 400×) SHOWING THIN WELL-FORMED EPIDERMIS AND NO INFLAMMATORY CELLS IN A WELL-ORGANIZED DERMIS

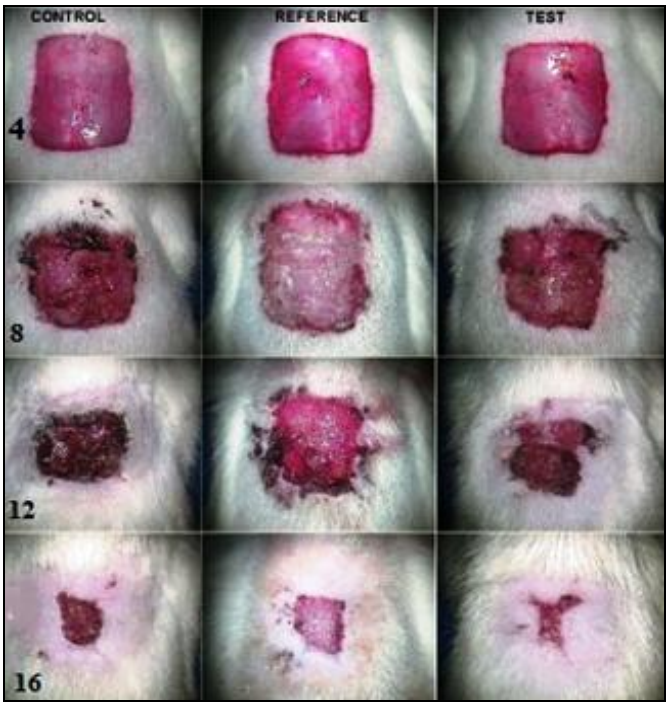

FIGURE 3: APPEARANCE OF WOUND HEALING IN DAY 4, 8, 12 &16

TABLE 1: EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON TENSILE STRENGTH IN INCISION WOUND MODEL

Group	Tensile strength (G ± SEM)
CONTROL	139.2±0.374
NFZ	187.4±1.887
SA 5%	190±2.235***+
SA 10%	220±0.687***+++xx

Values are expressed as Mean ± SEM (n=6); *(P<0.0001) Vs control group; +(P<0.0001) Vs NFZ group; x (P<0.0001) Vs SA 10%

Hydroxyproline and Proline play key roles for collagen stability. They permit the sharp twisting of the collagen helix. They help with providing stability to the triple-helical structure of collagen by forming hydrogen bonds. Hydroxyproline is found in few proteins other than collagen. The only other mammalian protein which includes hydroxyproline is elastin.

For this reason, hydroxyproline content has been used as an indicator to determine collagen content. Hydroxyproline contents were found to be increased significantly in the treated groups than the control group (**Table 3**), which implies more collagen deposition in the treated groups than the control group.

Studies on antioxidant enzymes revealed that the 10% treated group of *S. asoca* showed significant increase in the levels of superoxide dismutase, catalase, vitamin C, glutathione, the powerful antioxidant enzymes in the body that are known to quench superoxide radicals and there is a subsequent decrease in lipid peroxidation levels (**Tables 4, 5, 6, 7, 8, 9**).

TABLE 2: EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON WOUND CLOSURE AND EPITHELIALIZATION IN EXCISION WOUND MODEL

	% Of closure of excision wound area				
GROUPS	**DAY4**	**DAY 8**	**DAY 12**	**DAY 16**	**Epithelialization Period**
CTRL	29.4±2.159	56.8±0.663	73.8±2.354	85.2±1.772	21±0.774
NFZ	31.8±1.463	66.6±1.435***	84.2±1.393***	89.4±0.509	18.6±0.244
SA 5%	29.6±1.400	58.8±0.347 +++	75±0.078+++	88±0.326	19.9±0.210
SA 10%	32.1±2.630	69.3±0.960***xxx	86±0.004*** xxx	90.9±1.124**	18.8±1.548

Values are expressed as Mean ± SEM (n=6); ***(p<0.0001) Vs control group,**(p<0.0013) Vs control group,*(p<0.038) Vs control group; +++(p<0.0001) Vs NFZ group, ++(p<0.002) Vs NFZ group, +(p<0.0425) Vs NFZ group; xx (p<0.001) Vs SA 5%, x (p<0.045) Vs SA 5%

TABLE 3: EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON HYDROXYPROLINE CONTENT IN DEAD SPACE WOUND MODEL

Groups	Dry weight of granulation tissue	Hydroxyproline (µG/ML)	
		2nd Day	10th Day
Control	0.499 ± 0.14	1.078±0.308	4.348±1.032
NFZ	0.59 ± 0.130	3.942±0.211***	10.468±0.164***
SA 5%	0.535 ± 0.010	8.89±0.103***+++	12.305±0.150 ***
SA 10%	1.349 ± 0.208**++xx	10.082±0.600***+++	17.789±1.002***+++xxx

Values are expressed as Mean ± SEM (n=6); ***(p<0.0001) Vs control group; +++(p<0.0001) Vs NFZ group, ++(p<0.001) Vs NFZ group; xxx (p<0.0001) Vs SA 5%, xx (p<0.001) Vs SA 5%

TABLE 4 : EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON ENZYMATIC, NONENZYMATIC ANTIOXIDANTS AND ON LIPID PEROXIDATION IN INCISION WOUND MODEL ON SECOND DAY OF TREATMENT

Groups	SOD (IU/ml)	Catalase (µmoles/mg/min)	GSH (mg/dl)	Vit C (mg/dl)	Lipid Peroxidation (n mols/ml/hr)
Incision control	0.062±0.032	2.124±0.037	0.482±0.051	0.0494±0.004	0.088±0.003
Incision standard-NFZ	1.562±0.100	2.760±0.193	1.621±0.138	0.068±0.002	0.098±0.005
Incision treated- 5%	0.162±0.020	2.704±0.230	1.533±0.162	0.148±0.026**+	0.134±0.013**+
Incision treated- 10%	1.219±0.287***xxx	3.204±0.007***	1.895±0.011***	0.260±0.027***+++xx	0.142±0.010**+

Values are expressed as Mean ± SEM; n=6 in each group; *** Indicates ($p<0.001$) compared with control, ** Indicates ($p<0.01$) compared with control. +++ Indicates ($p<0.001$) compared with standard, + Indicates ($p<0.05$) compared with standard. xxx Indicates ($p<0.001$) compared with CE5%, xx Indicates ($p<0.01$) compared with CE5% .

TABLE 5: EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON ENZYMATIC, NONENZYMATIC ANTIOXIDANTS AND ON LIPID PEROXIDATION IN INCISION WOUND MODEL ON SEVENTH DAY OF TREATMENT

Groups	SOD (IU/ml)	Catalase (µmoles/mg/min)	GSH (mg/dl)	Vit C (mg/dl)	Lipid Peroxidation (n mols/ml/hr)
Incision control	0.178±0.02	2.166±0.024	0.520±0.040	0.064±0.007	0.082±0.003
Incision standard-NFZ	2.252±0.20	2.928±0.163	1.655±0.141	0.082±0.003	0.089±0.004
Incision treated- 5%	0.342±0.01	3.121±0.007	1.563±0.166***	0.201±0.006***+++	0.122±0.014*
Incision treated- 10%	2.197±0.04***xxx	3.305±0.022***+	2.085±0.048***x	1.024±0.021***+++xxx	0.119±0.009*

Values are expressed as Mean ± SEM; n=6 in each group. *** Indicates ($p<0.001$) compared with control, ** Indicates ($p<0.01$) compared with control, *Indicates ($p<0.05$) compared with control. +++ Indicates ($p<0.001$) compared with standard, + Indicates ($p<0.05$) compared with standard. xxx Indicates ($p<0.001$) compared with CE5%, x Indicates ($p<0.05$) compared with CE5% .

TABLE 6: EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON ENZYMATIC, NONENZYMATIC ANTIOXIDANTS AND ON LIPID PEROXIDATION IN EXCISION WOUND MODEL ON SECOND DAY OF TREATMENT

Groups	SOD (IU/ml)	Catalase (µmoles/mg/min)	GSH (mg/dl)	Vit C (mg/dl)	Lipid Peroxidation (n mols/ml/hr)
Excision control	0.082±0.04	2.145±0.023	0.512±0.061	0.051±0.004	0.089±0.003
Excision standard-NFZ	1.792±0.33	2.777±0.199	1.77±0.066	0.071±0.002	0.1±0.005
Excision treated-5%	0.194±0.02	2.756±0.231***	1.587±0.160 ***	0.151±0.026 ** +	0.136±0.013*
Excision treated-10%	1.482±0.15***xxx	3.242±0.029***	1.94±0.010***	0.264±0.027***+++xx	0.145±0.010**+

Values are expressed as Mean ± SEM; n=6 in each group; *** Indicates ($p<0.001$) compared with control, ** Indicates ($p<0.01$) compared with control, *Indicates ($p<0.05$) compared with control. +++ Indicates ($p<0.001$) compared with standard, + Indicates ($p<0.05$) compared with standard. xxx Indicates ($p<0.001$) compared with CE5%, xx Indicates ($p<0.01$) compared with CE5% .

TABLE 7: EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON ENZYMATIC, NONENZYMATIC ANTIOXIDANTS AND ON LIPID PEROXIDATION IN EXCISION WOUND MODEL ON TENTH DAY OF TREATMENT

Groups	SOD (IU/ml)	Catalase (µmoles/mg/min)	GSH (mg/dl)	Vit C (mg/dl)	Lipid Peroxidation (nmols/ml/hr)
Excision control	0.106±0.041	2.191±0.022	0.539±0.050	0.066±0.006	0.083±0.003
Excision standard-NFZ	2.2±0.470	3.077±0.044	1.824±0.053	0.084±0.003	0.090±0.004
Excision treated- 5%	0.234±0.022	3.077±0.032***	1.643±0.169 ***	0.204±0.006 ** ++	0.122±0.014*
Excision treated- 10%	1.851±0.324***xxx	3.326±0.027***+++xxx	2.023±0.021***x	1.068±0.043***+++xxx	0.119±0.009

Values are expressed as Mean ± SEM; n=6 in each group; *** Indicates ($p<0.001$) compared with control, ** Indicates ($p<0.01$) compared with control, *Indicates ($p<0.05$) compared with control. +++ Indicates ($p<0.001$) compared with standard, ++ Indicates ($p<0.01$) compared with standard. xxx Indicates ($p<0.001$) compared with CE5%, x Indicates ($p<0.05$) compared with CE5% .

TABLE 8: EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON ENZYMATIC, NONENZYMATIC ANTIOXIDANTS AND ON LIPID PEROXIDATION IN DEAD SPACE WOUND MODEL ON SECOND DAY OF TREATMENT

GROUPS	SOD (IU/ml)	Catalase (µmoles/mg/min)	GSH (mg/dl)	Vit C (mg/dl)	Lipid Peroxidation (n mols/ml/hr)
Excision control	0.084±0.019	2.148±0.022	0.504±0.064	0.053±0.00	0.072±0.015
Excision standard-NFZ	1.642±0.140	2.801±0.206	1.630±0.140	0.073±0.00	0.101±0.005
Excision treated-5%	0.173±0.019	2.770±0.213**	1.552±0.158 ***	0.153±0.025 ** +	0.137±0.013**
Excision treated-10%	1.266±0.294***xx	3.249±0.028***	1.89±0.018***	0.266±0.02***+++xx	0.146±0.010**

Values are expressed as Mean ± SEM; n=6 in each group; *** Indicates ($p<0.001$) compared with control, ** Indicates ($p<0.01$) compared with control. +++ Indicates ($p<0.001$) compared with standard, + Indicates ($p<0.05$) compared with standard. xx Indicates ($p<0.01$) compared with CE5% .

TABLE 9: EFFECT OF CHLOROFORM EXTRACT OF *S. ASOCA* ON ENZYMATIC, NONENZYMATIC ANTIOXIDANTS AND ON LIPID PEROXIDATION IN DEAD SPACE WOUND MODEL ON SEVENTH DAY OF TREATMENT

GROUPS	SOD (IU/ml)	Catalase (µmoles/mg/min)	GSH (mg/dl)	Vit C (mg/dl)	Lipid Peroxidation (n mols/ml/hr)
Excision control	0.185±0.026	2.172±0.023	0.533±0.070	0.072±0.006	0.069±0.015
Excision standard-NFZ	2.286±0.188	3.156±0.050	1.78±0.093	0.084±0.004	0.099±0.005
Excision treated- 5%	0.356±0.017	3.128±0.006***	1.698±0.108 ***	0.210±0.007 ***+++	0.135±0.138**
Excision treated- 10%	2.242±0.031***xxx	3.299±0.011***+ xx	2.035±0.028 ***	1.118±0.033***+++xxx	0.144±0.010**

Values are expressed as Mean ± SEM; n=6 in each group; *** Indicates ($p<0.001$) compared with control, ** Indicates ($p<0.01$) compared with control. +++ Indicates ($p<0.001$) compared with standard. xxx Indicates ($p<0.001$) compared with CE5%, xx Indicates ($p<0.01$) compared with CE5% .

DISCUSSION: The preliminary Phyto-chemical screening of the chloroform extract of *S. asoca* showed the presence of carbohydrates, steroids, alkaloids, glycosides. They are responsible for their wound healing and anti-oxidant property.

Wound healing is a step wise process, which consists of different phases such as haemostasis, inflammation, proliferation, remodelling and maturation phase. The three different models were used in the present study to assess the wound healing activity of chloroform extract of *Saraca asoca*. The standard drug nitrofurazone is used to assess the healing potency of crude drugs . Plants are the storehouses for the variety of phytochemical constituents. The process of healing is promoted by various active principles like triterpenes, alkaloids, flavonoids [20].

Increase in breaking strength of NFZ-ointment treated animals showed improved collagen migration by increased cross linking. Wound contraction is defined as the centripetal movement of the edges of a full thickness wound in order to promote seal of the defect. The rate of wound contraction was less in control and standard groups when compared to SA-ointment treated animals increase in breaking strength of *S. asoca* ointment treated animals showed improved collagen migration by cross linking.

Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema & new small blood vessels. Collagen is a major compound that strengthens extracellular tissue and is composed of amino acids, hydroxylproline, which is used as a biomarker for tissue collagen. In the present study hydroxyproline estimation is increased levels in 10% treated groups and also there is a significant increase in the granulation tissue.

The wound healing activity of this medicinal plant is attributed to the active constituents present in it . Alkaloids, glycosides are responsible for their antimicrobial property. Glycosides are reported to be responsible for wound healing activity in *Leucas hirta, Ocimmum sanctum, gratissimum.* Proteins, carbohydrates provide essential nutrients for proper healing of wound [21]. Plant products are shown to possess good therapeutic potential as anti-inflammatory agents and graduate wound healing, due to the presence of active terpenes, steroids, alkaloids and flavanoids [22]. The cardiac glycosides exhibited antioxidant and antimicrobial properties in various plant studies [23, 24.] Steroids have anti-bacterial and antioxidant potential [25, 26].

Antioxidant activity favours the regeneration of wounds, because the production of reactive oxygen species during the process of tissue injury aggravates the disorders in the tissue. As free radicals cause damage to membrane lipids, proteins, enzymes, nucleic acids hence, scavenging effect might be one of the essential components of wound healing. Enzymatic antioxidants (Superoxide dismutase (SOD), Catalase) and non-enzymatic antioxidants (Glutathione (GSH), Vitamin C) are known to quench radicals and thus prevent the damage of cells [27]. Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. By products of lipid peroxidation causes marked damage of cell structure and finally results in functional disruption. These byproducts formed under physiological and pathological conditions are scavenged by enzymatic and non-enzymatic antioxidants.

The steroids, alkaloids, cardiac and anthraquinone glycosides of *S. asoca* promotes the process of wound healing by increasing the viability and strength of collagen fibres, either by increasing the circulation or by preventing the cell damage or by promoting the DNA synthesis.

CONCLUSION: Phytochemical constituents present in *S. asoca* may be responsible for the wound healing activity. As *S. asoca* is having antibacterial and anti-oxidant property it could be advantageous for the treatment of wound infections.

This study shows that *S. asoca* has wound healing effect when formulated as ointment and could therefore explain the success sores, boils and wounds.

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