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Exploring the Biological potential of Marine Macroalgae

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INTRODUCTION

The most frequent and dangerous conditions that diabetes individuals experience are wound infections.[1] One of the main causes of physical disability is injury.[2] The Wound Healing Society defines wounds as physical injuries that result in a break in the skin or an opening in the skin that disturbs the normal functioning and structure of the skin.[3] The intricate interplay of cellular and biochemical processes leading to the recovery of wounded tissues' strength and structural and functional integrity is known as wound healing. Inflammation, wound contraction, reepithelialization of the tissue, remodelling, and the creation of granulation tissue with angiogenesis are among the many overlapping phases and processes involved.[4] entails an acute phase of inflammation at first, which is followed by the formation of extracellular macromolecules such as collagen, and eventually creates a scar. *Spongomorpha india* is a macroalgae belonging to the family *ulotrichaceae*.[5] Literature review reveals the presence of antibacterial, antifungal, antiviral, anti-inflammatory activities. The macroalgae *Spongomorpha india* is a member of the *ulotrichaceae* family. A study of the literature indicates the existence of antiviral, antibacterial, antifungal, and anti-inflammatory properties. The research also suggests that there isn't any scientific proof for *Spongomorpha indica's* anti-diabetic model or its ability to repair wounds in delayed-onset diabetes models. In order to determine the scientific foundation for the use of algae in the treatment of antidiabetic activity and wound healing impact on rats induced with diabetes, the current study examines the action of hydroalcoholic *Spongomorpha indica* (HASI) extract in several rat models.

MATERIAL AND METHODS

Collection and Preparation of extract

The marine macroalgae was taken off the beach and stuck to the rocks. It was first properly cleaned with sea water, then with distilled water, and dried in the shade. Any excess material

was sieved out. Following the drying process, the product was coarsely ground and extracted using hydro-alcohol 70:30 (70% v/v methanol) in three separate extractions utilizing maceration chambers. The result was then vacuum-filtered to eliminate any remaining fine particles, trash, dust, etc. Following the extraction process, the filtrates were dried, kept in a desiccator, and concentrated using a distillation unit.

Experimental animals

Two breeds of rats, Swiss Albino mice (25–30 g) and Wistar rats (150–200 g), were used in all of the studies. Paddy husk was used as the bedding basis in polypropylene cages where the animals were arranged at random. Every animal was kept in a room with a temperature of 24 ± 2°C and a relative humidity of between 30 and 70%. There was a 12-hour day and 12-hour night cycle. Every animal was given unrestricted access to food and water. The Institutional Animal Ethical Committee provided ethical approval to conduct this experiment under Registration No. 527/PO/ReBi-S/07/IAEC.

Acute toxicity study

In accordance with OECD 423 Guidelines, the acute toxicity investigation was conducted. For the experiment, Wistar albino mice weighing between 25 and 30 grams were selected. The animals were divided into three groups, and each group received a different dosage of Spongomorpha indica hydro-alcoholic extract based on its body weight (mg/kg). After the first animal received a starting dose of 5 mg/kg and no deaths were noted, the next group of animals received even higher doses. For 14 days, the animals were watched to look for any indications of delayed toxicity.[6]

Glucose tolerance test[7]

Rats that had fasted for the night were split up into five groups, each with six rats. Group I was given 2 milliliters of distilled water to serve as a control, Group II was given only glucose, Group III was given the standard medication Glibenclamide at a dose of 5 milligrams per kilogram body weight, Groups IV and V were given 200 mg and 400 mg, respectively, of the hydro-alcoholic extract of Spongomorpha indica (HASI) extract.

With the exception of Group I, all rats in Groups III, IV, and V received oral treatment with 2 g/kg of glucose after 30 minutes of delivery. Rat blood samples were taken from the tail vein in each group immediately before glucose was administered as well as 30, 60, 90, and 120 minutes later. With the aid of a digital glucometer (one touch choose, Johnson & Johnson, USA), blood glucose levels were determined right away.

Streptozotocin induced anti-diabetic activity

Induction of diabetes

Before practice, fresh STZ was made by dissolving in citrate buffer (0.01M, PH-4.5) and storing it on ice. To induce diabetes, a single intraperitoneal injection of STZ (60 mg/kg) was given to the rats who had fasted overnight. Five percent glucose was given orally in drinking water for a day following the four hours of STZ treatment in order to overcome the early hypoglycemia phase. Three days were given to the rats to stabilize. Blood samples were taken three days later, or after 72 hours, to measure the blood glucose level and validate the onset of diabetes.[3] Rats classified as diabetic if their plasma glucose level was more than 250 mg/dl and measured using a digital glucometer (Accu-Chek, Roche Diabetes Care India) were employed in the investigation.

Group 1 served as the control, Group 2 as the diabetic control, Group 3 as the standard (glibenclemide 5 mg/kg), Group 4 as the sample with 200 mg HASI given orally for 14 days, and Group 5 as the 400 mg HASI provided for 14 days. The animals were separated into five groups of six rats each. The SOD and Catalase models were used to calculate the body weights, blood glucose, urine glucose, and biochemical parameters such as VLDL, HDLP, triglycerides, and antioxidant properties.[8,9]

Dexamethasone induced diabetic model[7]

30 overnight fasted rats were chosen. One daily injection of Dexamethasone (10 mg/kg, s.c.) was used to make 24 rats diabetic. Group I was the normal control and was given distilled water; Group II was the diabetic control; Group III was the standard treatment and was given oral Glibenclamide at a dose of 5 mg/kg for 21 days; Group IV was the treatment group and was given 200 mg/kg of hydro-alcoholic extract of Spongomorpha indica (HASI) orally for 21 days; and Group V was the treatment group and was given 400 mg/kg of hydro-alcoholic extract of Spongomorpha indica (HASI) orally for 21 days. The results were ascertained by monitoring the body weights, blood glucose levels, urine glucose levels, and biochemical markers.

EFFECT OF *SPONGOMORPHA INDICA* EXTRACT IN WOUND HEALING DEXAMETHASONE INDUCED DIABETIC RATS

Experimental study design

Wistar albino rats weighing between 150 and 200 grams were chosen for this investigation, and the rodents were split into 6 groups, each with 4 rats. Dexamethasone 10 mg/kg/s.c. was given to the first group, which served as the control. The second group received the same dosage plus 5 mg/kg of Glibenclamide orally along with a topical application of Mupirocin ointment. The third group received the same dosage plus HASI 200 mg/kg orally, and the fourth group received the same dosage plus HASI 400 mg/kg orally.

Dosing schedule

Dexamethasone subcutaneously was given on alternate days (day 0 to 28 postoperative day) and *Spongomorpha indica* extract was given orally from day 0 to day 28 along with distilled water.

Excision wound[10]

The rats in this investigation were put to sleep with a dose of 25 mg/kg of thiopentone sodium intraperitoneally. The anesthetized rat was placed in an imprint on the dorsal thoracic area, which was placed 1 cm from the vertebral column and 5 cm from the ear. That specific area's skin was shaved before the experiment. A 500 mm2 wound was created by excising the skin of the impressed region to its maximum thickness. By blotting the wound with a cotton swab soaked in regular saline, hemostasis was achieved. Under the experimental design, animals

were given daily medication treatment from the 0th to the 28th day after the wound. On the 28th day following the wound, the wound area is measured. The wound's contraction and its percentage were computed. When a scar forms and no longer shows signs of a raw wound, it is said to have reached full epithelization. The number of days needed to reach this point is known as the epithelization period.

Incision Wound Model

Under anesthesia, a paravertebral straight incision of 6 cm was performed on each group of rats, using a sharp scapel to cut through the full thickness of the skin. Following total homeostasis, the incision was sealed with interrupted sutures spaced around 1 cm apart. Under the experimental design, animals were given daily treatment with the medications listed above from the 0th to the 28th day after the wound. On the 28th day, a tensiometer was used to evaluate the wound breaking strength.

The ability to withstand breaking under stress is known as tensile strength. It may also provide some insight into the quality of the restored tissue by indicating the degree to which the mended tissue resists breaking under strain. A tensiometer, which measures tensile strength, was used for this purpose by excising the freshly regenerated tissue, including the scar. One edge of the wound was fixed for quantification, while the other was subjected to a quantifiable force. On the tenth day following surgery, the incision had fully healed before determining the load, or weight, in grams needed to cause disruption.[11,12]

Dead space wound model[13,14]

Rats under anesthesia were given a subcutaneous injection of 10 mg of sterile cotton in the lumber area on the dorsal side to generate this kind of wound. Under the experimental design, animals were given daily treatment with the medications listed above from the 0th to the 28th day after the wound. Granulation tissue collected from the implanted cotton was carefully removed on the 28th post-wound day, dried for 24 hours at 60 ºC, and then weighed. At last, granulated tissue was examined histologically.

Hydroxyproline Estimation

On day 28, the hydroxyproline content was measured in granulation tissue that had been removed from both normal and diabetic-induced delayed wound model mice. Diethyl ether was used to put each group of rats to sleep so that the amount of hydroxyproline could be measured. The methods outlined by Neuman and Logan were used to estimate the tissue's protein concentration. The weight of the removed granulation tissue was noted. After the tissue was dried for 12 hours at 60 °C in an oven, the dry weight was once more recorded. They were hydrolyzed in sealed glass tubes with 6N HCl for 24 hours at 110 ◦C. The pH of the hydrolysate was brought to 7.0. First, 200µl of the samples were combined with 1 milliliter of 0.01M $CuSO_4$, then 1 milliliter of 2.5N NaOH and 1 milliliter of 6% H_2O_2. For five minutes, the solution was combined and sporadically shook. For five minutes, all of the tubes were incubated at 80°C with frequent, vigorous shaking. After cooling, 4 milliliters of 3N H_2SO_4 were vigorously added. Lastly, 2 milliliters of p-dimethylaminobenzaldehyde (5%), were added. A digital photo colorimeter (EI Products, India) was used to measure the absorbance of the samples at 500 nm after they had been incubated for 16 minutes at 70 ◦C and cooled by submerging the tubes in water at 20 ◦C. Using a standard curve made with pure L-hydroxyproline at the same time, the quantity of hydroxyproline in the samples was determined.[15]

Histopathological Studies

In the histology investigations, fragments of granulation tissues from dead space wound models were dried using an ethanol-xylene series of solutions after being fixed for 24 hours in 10% neutral formalin solution. Filtered and paraffin-embedded samples were used (40–60 °C). Ten G thick microtome slices were obtained. The sections were stained with hematoxylin-eosin dye after being treated in an alcohol-xylene series. Changes in the histology were seen under a microscope. Under a light microscope, slides were qualitatively inspected for the development of collagen, fibroblast proliferation, angiogenesis, epithelization, and granulation tissue.

Statistical analysis

Using Graph Pad Instat 5.0 software, a one-way analysis of variance (ANOVA) and Dunnett's technique of multiple comparisons were used. It was determined that $p<0.05$, $p<0.01$, and $p<0.001$ were statistically significant.

Results:

Acute oral toxicity study

Table 1: Acute oral toxicity study of hydro-alcoholic extract of *Spongomorpha indica* L *in* mice.

S. no.	Extracts	LD_{50} mg/kg	ED_{50} mg/kg
1	Hasi	2000	200

Table 2: Observation parameters in acute toxicities of hydro-alcoholic extract *Spongomorpha indica* L.

Parameters	Observation
Tremors	Not observed
Convulsions	Not observed
Salivation	Normal
Sleep	Normal
Diarrhoea	Feces normal
Lethargy	Observed laziness
Skin and fur	Normal
Eyes and mucous membrane	Normal
Respiratory	Normal
Circulatory	Normal
Autonomic and central nervous system	No observed changes
Somatomotor activity	Normal motor activity

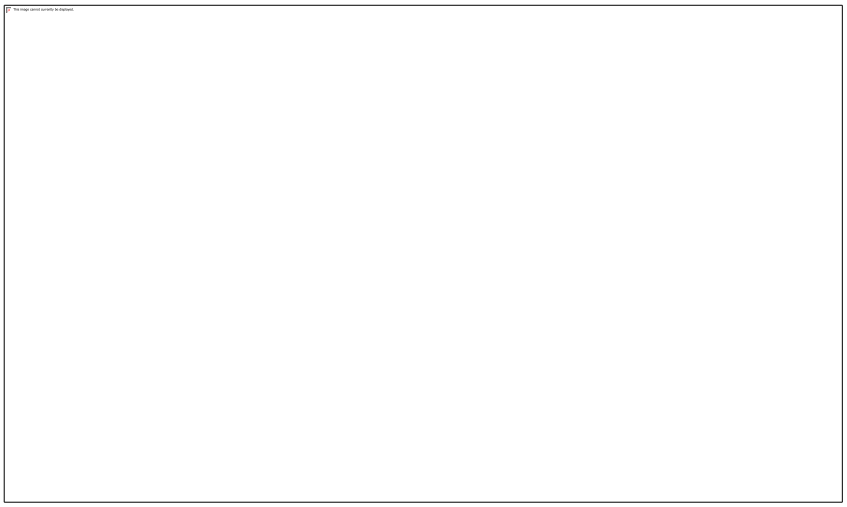
There was no mortality and toxicity was observed up to 2000mg/kg and the study was carried out with $1/10^{th}$ of LD_{50} of extract as 200 mg/kg Therapeutic dose (TD) and double the TD as 400 mg/kg

Table 3: Effects of *Hydro-Alcoholic extract Spongomorpha indica* L on oral glucose tolerance in rats.

Groups	0 min	30 min	60 min	90 min	120 min
Group I	80.22±0.85	79.23±1.25***	83.57±0.94	82.21±1.29***	80.56±3.12***
Group II	86.58±0.19	168.74±2.37	215.08±1.83	196.25±2.46	185.43±2.57
Group III	75.16±0.57	139.57±4.25***	150.28±0.84	129.68±1.43***	101.28±1.45***
Group IV	78.23±0.83	145.64±2.61***	158.29±1.85***	140.51±1.28***	129.54±2.17**
Group V	80.84±0.43	143.28±3.16***	156.53±2.57***	138.43±2.31***	101.61±1.84***

Values are expressed as Mean±SEM, n=6. Significant (***p <0.001) compared with treated groups Vs diabetic control.

Figure 1: Effects of *Hydro-Alcoholic Extract Spongomorpha indica* L on oral glucose tolerance in rats.



Streptozotocin induced diabetic rats

Table 4: Effect of *Spongomorpha indica* L on body Weight & Urine glucose.

		Body weight	Urine Glucose

Groups	Treatment	0th day	14th day	0th day	14th day
Group I	2 ml of Distilled water	176.48±2.07	212.35±2.59**	81.39±2.34	79.48±1.09***
Group II	Streptozotocin 60 mg/kg	161.66±1.47	178.83±1.24	87.24±1.96	375.46±0.83
Group III	glibenclamide (5mg/kg)	173.33±2.07	215.64±2.00**	78.15±2.74	128.91±1.37***
Group IV	200 mg/kg	180.83±1.38	227.73±1.77*	80.26±2.12	174.62±1.52**
Group V	400 mg/kg	174.16±1.74	216.61±1.07**	83.12±1.94	132.83±1.09***

The values are mean±SEM, n=6 when treated group compared with diabetic control $p<0.05^{*}$, **p<0.01, ***p<0.001.

Figure 2: Effect of *Spongomorpha indica* L on body weight.

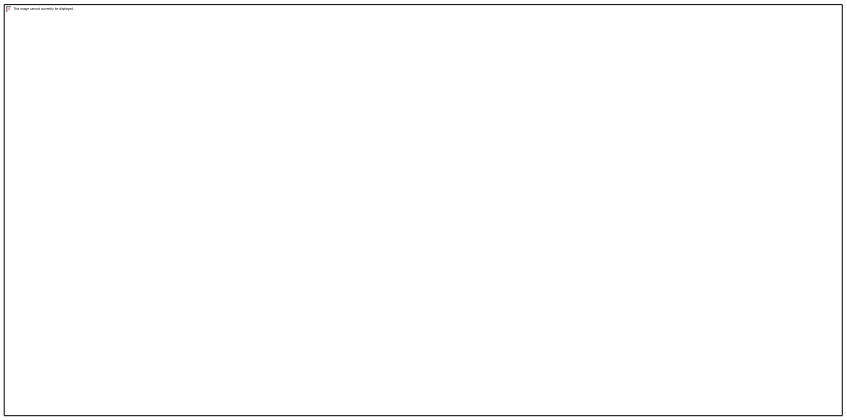


Figure 3: Effect of *Spongomorpha indica* L on urine glucose

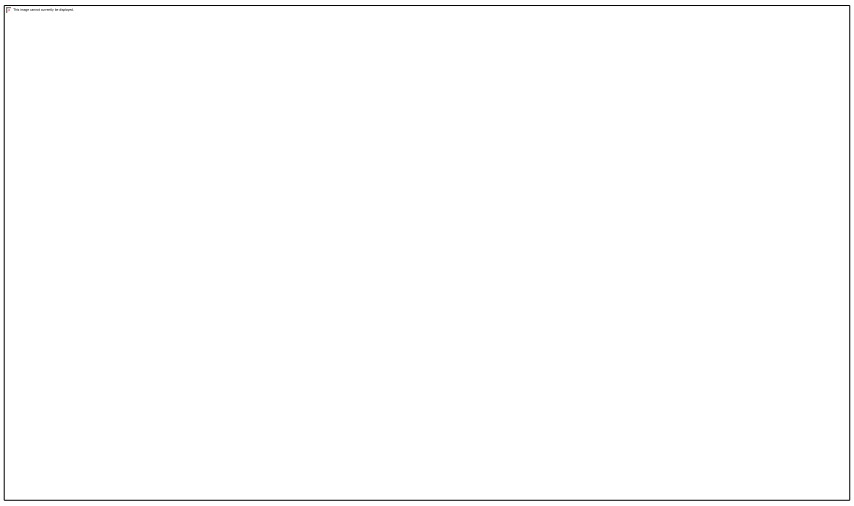


Table 5: Effect of *Spongomorpha indica* L on blood glucose level on streptozotocin induced diabetic rats.

Groups	Blood glucose level in mg/dl					
	0th day	**3rd day**	**6th day**	**9th day**	**11th day**	**14th day**
Group I	83.05±1.83	83.8±2.32***	76.63±1.52***	79.83±1.39***	80.60±1.79***[a]	82.84±1.05***[a]
Group II	85.5±1.1	382.16±2.9[c]	424.33±1.65[c]	451.66±1.5[c]	486.32±1.82[c]	500.00±1.50[c]
Group III	80.50±1.01	271.88±3.35**	235.0±1.9***	171.7 ±2.38***	142.0±1.39***[b]	110.0±1.42***[a]
Group IV	82.20±1.8	357.88±4.32**	315.0±2.32**	300.7 ±2.6***	220.0±1.12***[d]	174.10±1.02**[d]
Group V	81.24±1.21	278.92±3.45**	232.0±1.80***	187.8 ±1.88***	158.20±1.87***[b]	130.20±1.15***[b]

The values are mean±SEM, n=6 when compared with diabetic control *p<0.05, **p<0.01,***p<0.001. Mean bearing different superscripted differ significantly.

Figure 4: Effect of *Spongomorpha indica* L on blood glucose level on

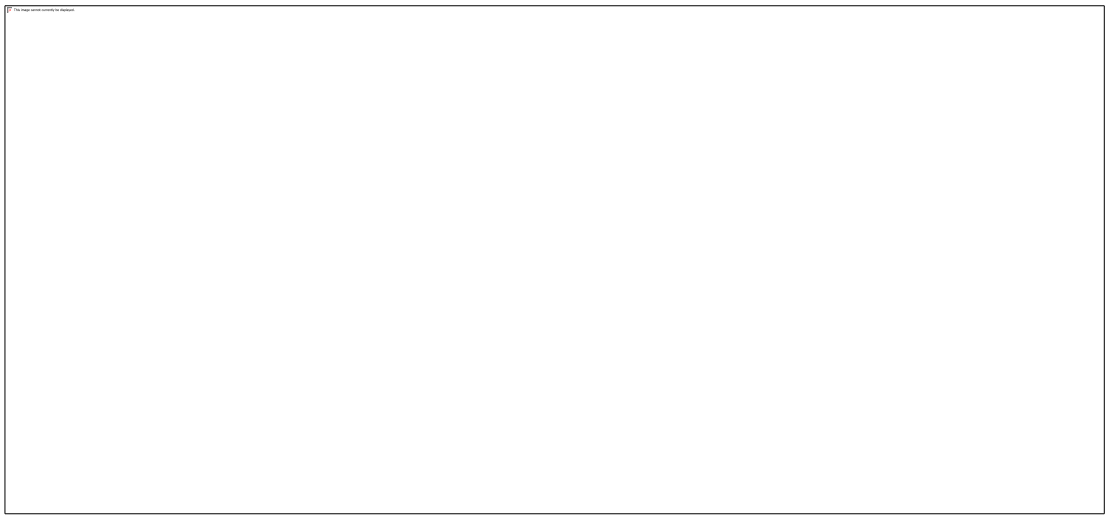
streptozotocininduced diabetic rats.

Table 6: Effect of *Spongomorpha indica* L on lipid parameters by streptozotocin induced diabetic rats.

Groups	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group I	137.47±1.76***	104.25±2.42***	37.83±1.72**	47.00±2.80***	12.65±1.27***
Group II	284.41±1.16	169.81±2.11	21.5±1.4	109.30±1.83	30.33±1.22
Group III	146.88±2.93***	118.86±1.42*	38.33±2.72**	48.67±1.28***	13.98±1.12***
Group IV	179.00±2.33**	152.56±1.33^{ns}	36.67±1.26**	75.25±1.52***	19.10±1.42**
Group V	142.64±1.05**	134.87±1.51*	34.83±1.94*	48.33±1.37***	12.35±1.15***

The values are mean±SEM, n=6 when treated groups compared with diabetic control *p<0.05, **p<0.01 & ***p<0.001.

Figure 5: Effect of *Spongomorpha indica* L on lipid parameters by streptozotocin induced diabetic rats.



Anti-oxidant study

Group II animals exhibited decreased levels pf protective antioxidant enzymes such as SOD and CAT, suggesting a possible free radicals generation. Treatment with standard, HASI 200 & 400 MG/Kg SHOWED SIGNIFICANTLY (P<0.01) increased levels of protective enzymes such as SOD AND CAT, suggesting its possible antioxidant action. Whereas group III revealed less significant changes was observed compared to extract treated group.

Table 7: Effect of *Spongomorpha indica* L extract on Anti-oxidant enzyme inStreptozotocin induced diabetic rats.

Groups	Treatment	SOD (U/mg of tissue)	CAT (U/mg of tissue)
Group I	Normal Control	6.25±0.02	10.60±0.4
Group II	Streptozotocin	1.60±0.04	3.82±0.20
Group III	Standard	3.12±0.05*	6.01±0.46*
Group IV	HASI 200 mg/kg	4.44±0.01**	7.92±0.29**
Group V	HASI 400 mg/kg	5.64±0.06**	9.55±0.55**

The values are mean±SEM, n=6 when treated group compared with diabetic control *p<0.05, **p<0.01.

Figure 6: Effect of *Spongomorpha indica* L extract on Anti-oxidant enzyme in Streptozotocin induced diabetic rats.

Histopathology of pancreas in streptozotocin induced diabetic rats

Normal control rat pancreas with typical Langerhans islets containing pale, ovoid, rounded β-cells embedded in the exocrine part of the pancreas. In the pancreas of a diabetic control rat, the islets of Langerhans have shrunk and there is clear karyolysis and degeneration of the constituent cells, where the nucleus has become heavily basophilic. The pancreas of a diabetic

rat given glibenclamide and 200 mg/kg of HASI exhibited normal Langerhans islets, which are big, pale, round to ovoid-shaped organs that contain cells embedded in the exocrine region of the pancreas. The pancreas of a diabetic rat given 400 mg/kg of HASI showed normal-sized islets of Langerhans, although there was some center-lobe β cell degradation.

Dexamethasone induced diabetic rats

Table 8: Effect of *Spongomorpha indica*

Groups	Treatment	Body weight		Urine Glucose	
		0th day	**21st day**	**0th day**	**21st day**
Group I	Normal Control	156.48±2.07	252.35±2.59**	80.51±1.90	83.27±1.17***
Group II	Dexamethazone 10 mg/kg	151.66±1.47	169.83±1.24	79.27±1.38	305.32±1.02
Group III	Glibenclamide (5mg/kg)	163.33±2.07	252.64±2.00**	78.54±1.65	116.71±1.07***
Group IV	HASI 200 mg/kg	170.83±1.38	227.73±1.77*	81.69±1.58	184.49±1.76**
Group V	HASI 400 mg/kg	174.16±1.74	245.61±1.07**	84.38±1.47	157.62±1.41***

The values are mean±SEM, n=6 when treated group compared with diabetic control $p<0.05^{*}$, **p<0.01, ***p<0.001

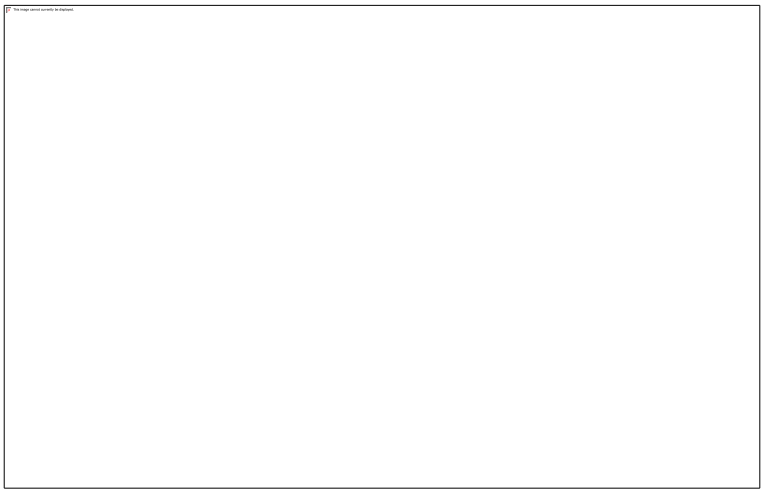


Figure 7: Effect of *Spongomorpha indica* L on body weight in dexamethasone induceddiabetic rats.

Figure 8: Effect of *Spongomorpha indica* L on urine glucose in dexamethasone induced diabetic rats.

Table 9: Theeffect of *Spongomorpha Indica* L extract on blood glucose level in dexamethasone induced hyperglycemic rats.

Groups	Blood glucose level in mg/dl							
	0 day	3 day	6 day	9 day	11 day	14 Day	17 Day	21 day
Group I	84.66± 4.88	89.33± 4.07***	80.16± 2.57***	101.7± 4.11***	97.7±1. 05***	85.45±4. 11***	82.34±2. 43***	86.5±1. 57***a
Group II	76.83± 5.26	248.16± 4.98	267.47 ± 5.17	286.33± 2.84	309.43± 3.48	345.28± 5.27	364.26± 3.79	380.54± 2.38
Group III	82.16± 2.27	178.5±4 00**	186.28 ± 4.06**	158.24±2. 51***	139.52±2. 31***	130.57±2. 51***	124.62± 2.19***	113.17± 2.81***a
Group IV	77.33± 3.21	195.5±3. 73*	204.64 ± 5.45*	195.74± 2.59***	176.70± 2.59***	152.45±3. 59***	144.91± 2.46***	122.92± 2.12***
Group V	83.83± 1.99	176.66± 2.72**	198.15 ± 2.90**	163.15± 3.12***	143.5±3. 04***	130.47±3. 04***	123.5±3. 04***	94.50+3 .75***a

The values are mean ± SEM, n=6 when compared with diabetic control **p<0.01

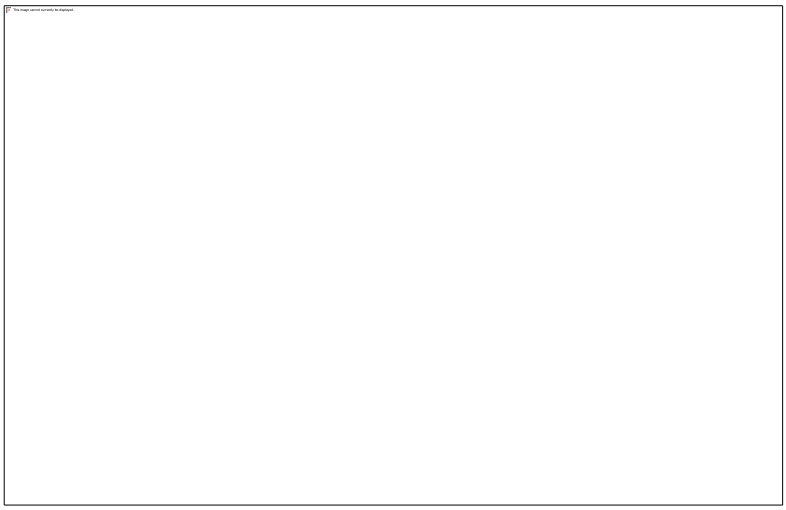


Figure 9: The effect of *Spongomorpha indica* L extract on blood glucose level in dexamethasone induced hyperglycemic rats.

Table 10: Effect of *Spongomorpha indica* L extract on lipidprofilein dexamethasone induced hyperglycemic rats.

Groups	TG (mg/dl)	TC (mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
Group I	118.47±3.13***[a]	153.18±4.27***	36.08±2.80**	42.13±2.24***	28.49±2.24***
Group II	235.79±5.25	238.27±4.91	24.01±1.05	68.75±3.38	69.13±4.25
Group III	139.50±3.00***[b]	162±3.52***	34.64±2.93***	49.43±2.36**	34.27±2.32***
Group IV	145.18±4.93***[c]	163.75±2.88***	32.53±2.14**	50.43±2.15*	36.26±2.62***
Group V	124.82±1.00***[a]	158.61±4.83***	38.59±2.25*	43.18±3.81***	30.46±2.83***

The values are mean±SEM, n=6 when treated groups compared with diabetic control *p<0.05, **p<0.01 &***p<0.001. Means bearing same superscript do not differ significantly between treated groups. Means bearing different superscript differ significantly at p<0.01 & p<0.05, between treated groups.

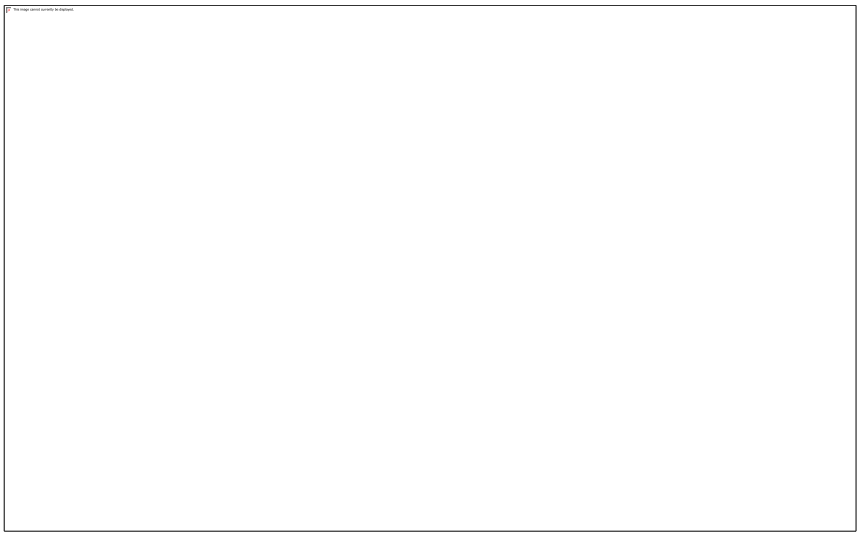


Figure 11: Effect of *Spongomorpha indica* L extract on lipidprofilein dexamethasone induced hyperglycemic rats.

DEXAMETHASONE INDUCED DELAYED WOUND HEALING MODEL

Table 8: Effect of *Spongomorpha Indica* L on by dexamethasone induced delayed Excision wound model on 28th day

Treatment Group	Wound contraction in mm^2	Scar area in mm^2	Epithelizationperiod in days	Percentage of wound contraction
Dexamethasone 10mg/kg	98.47±6.66	136.16±1.35	32.4±0.56	20.82±3.82
Dexa +GLB+Mupirocin	343.58±3.73**∗a	57.16±1.94**a	20.54±0.63**a	68.81±5.97***a
HASI 200 mg/kg	287.83±2.61**b	78.52±1.27*b	28.59±0.61*b	57.66±3.43**a
HASI 400 mg/kg	335.17±2.42**∗a	58.35±1.46**a	20.87±0.52**a	66.93±2.51***a

Values are Mean ± SEM from 6 animals in each group. Means bearing same superscript do not differ significantly betweentreated groups. Means bearing different superscript differ significantly between treated groups**.**

Figure 7: Effect of *Spongomorpha Indica* L on wound contraction in mm^2

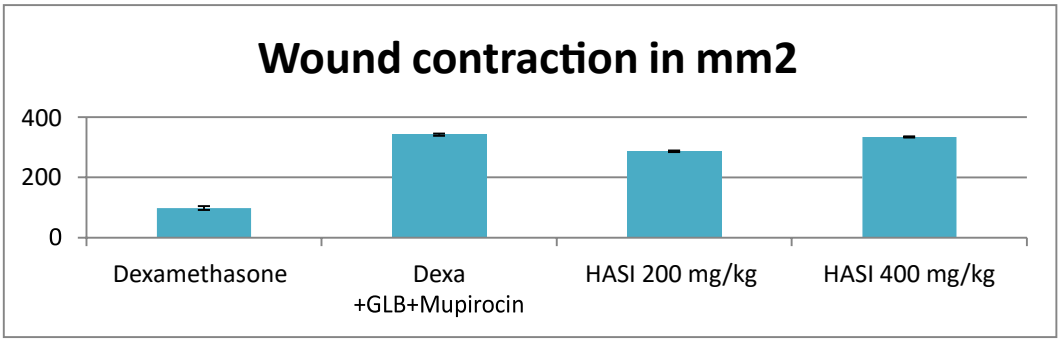


Figure 8: Effect of *Spongomorpha Indica* L on Scar area in mm^2

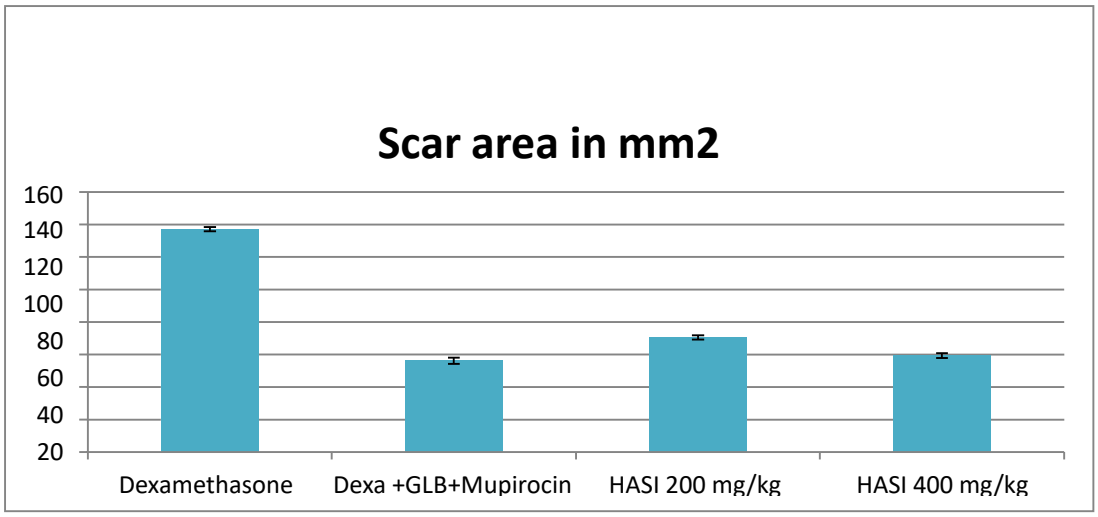


Figure 9: Effect of *Spongomorpha Indica* L on Epithelization period in days

Figure 10: Effect of *Spongomorpha Indica* L on Percentage of wound contraction

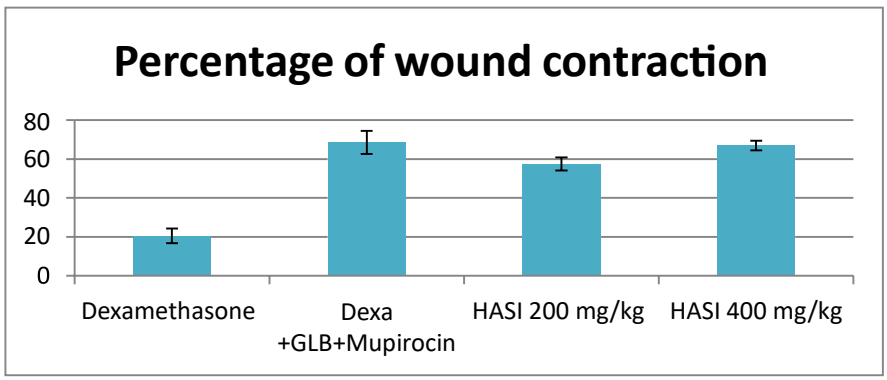
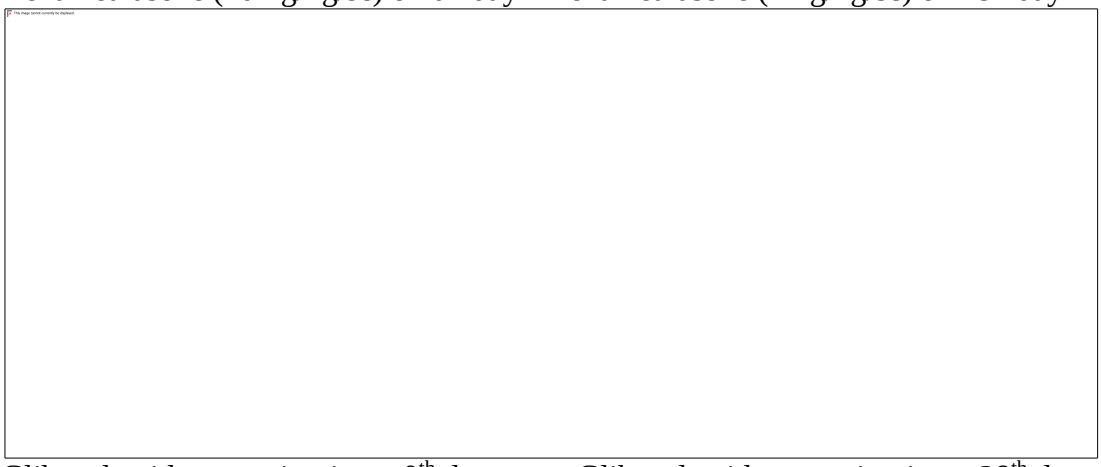
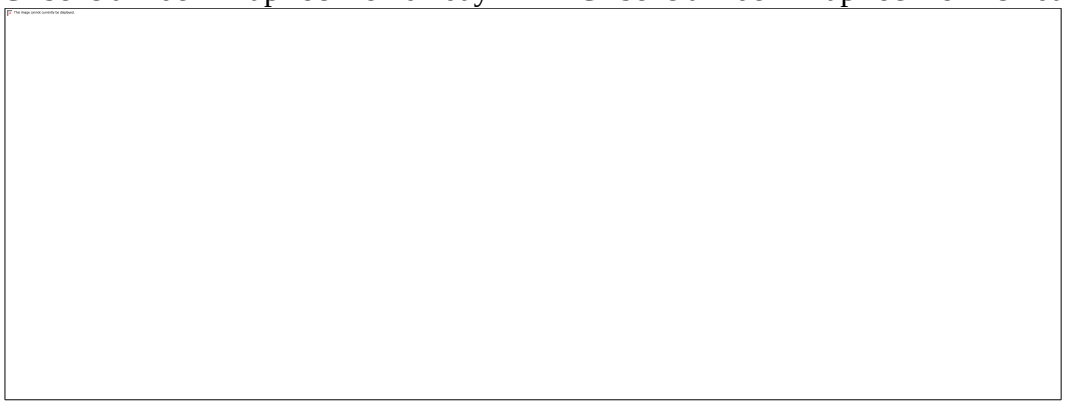


Figure 11: Effect of *Spongomorpha Indica* L extract on excision wound model in dexamethasone induced diabetic rats.

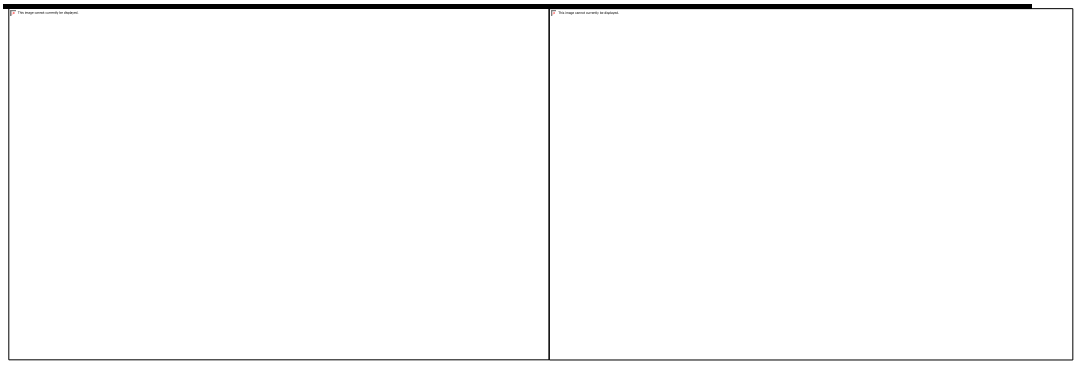
Dexamethasone (10mg/kg/sc) on 0th day Dexamethasone (4mg/kg/sc) on 28th day



Glibenclamide + mupirocin on 0th day Glibenclamide + mupirocin on 28th day



HASI 200 mg/kg on 0th day HASI 200 mg/kg on 28th day



HASI 400 mg/kg on 0th day HASI 400 mg/kg on 28th day

Table II: Effect of *Spongomorpha Indica* L extract on tensile strength in dexamethasone induced delayed wound healing, incision wound model on 28th day.

Table 9:

Treatment Group	Tensile strength/ Breaking strength
Dexamethasone 10mg/kg/sc	157.45±1.64
Dexa +GLB+Mupirocin	269.48±3.57***[a]
HASI 200 mg/kg	224.39±2.83**[b]
HASI 400 mg/kg	256.94±3.26***[a]

Values are Mean ± SEM from 6 animals in each group. Significant difference at $p<0.01$ & $p<0.05$ when treated groups compared to dexamethasone control. Means bearing same superscript do not differ significantly between treated groups. Means bearing different superscript differ significantly.

Figure 12: Effect of *Spongomorpha Indica* L extract on Tensile strength in dexamethasone induced delayed wound healing, incision wound model on 28th day

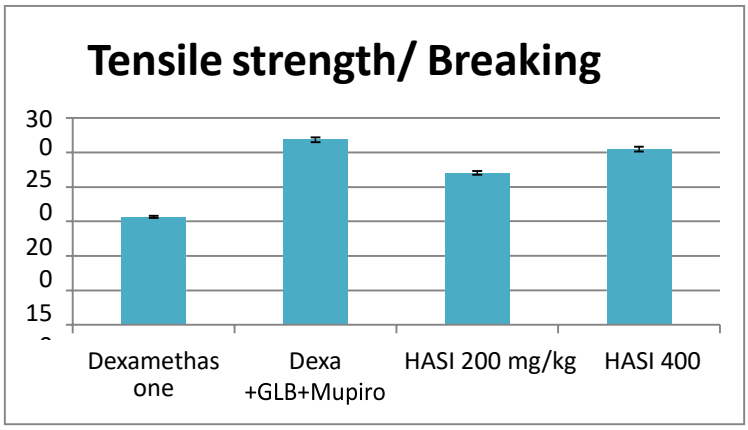
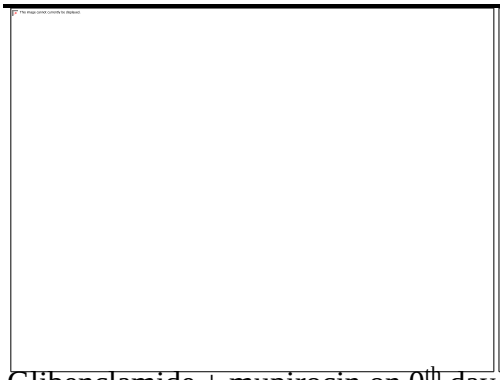
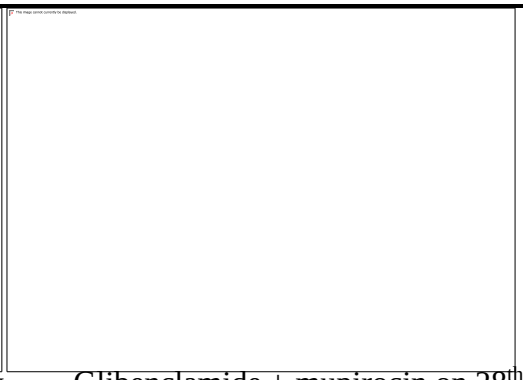


Figure 13: Effect of *Spongomorpha Indica L extract* on incision wound model in dexamethasone induced diabetic rats.

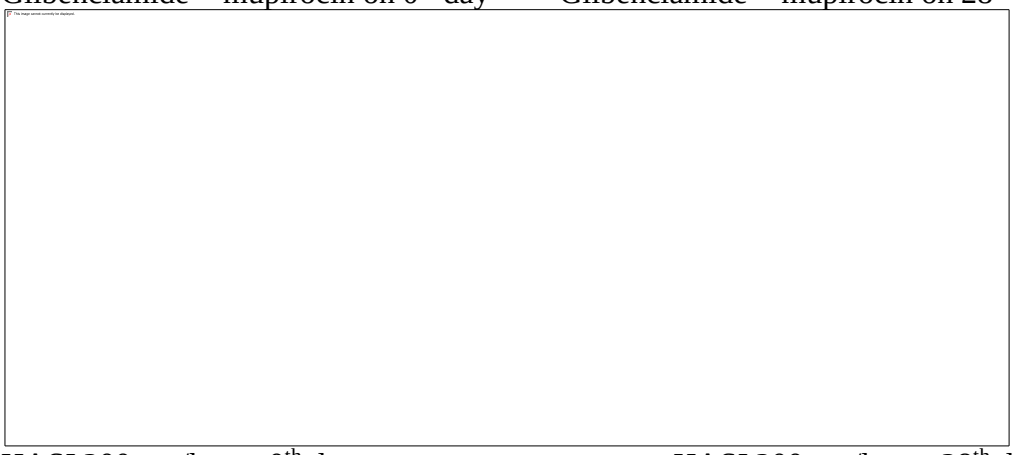
Dexamethasone (10mg/kg/sc) on 0th day Dexamethasone (4mg/kg/sc) on 28th day



Glibenclamide + mupirocin on 0^{th} day

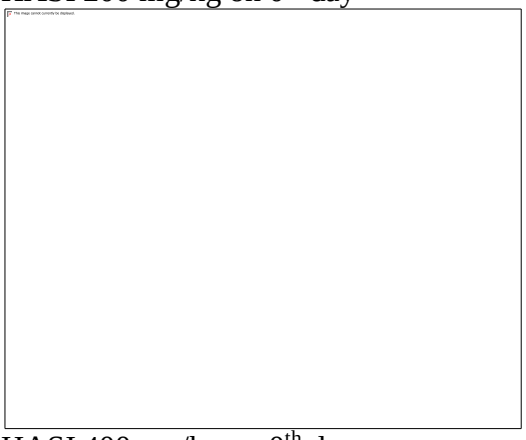


Glibenclamide + mupirocin on 28^{th} day

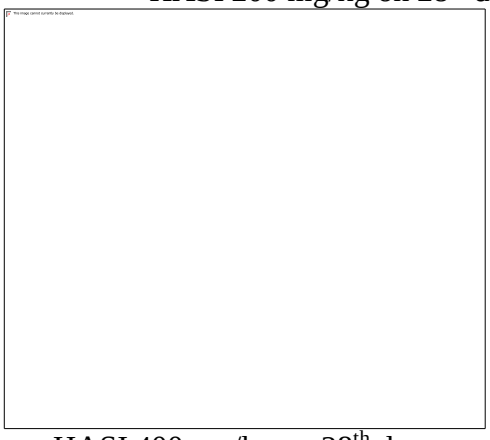


HASI 200 mg/kg on 0^{th} day

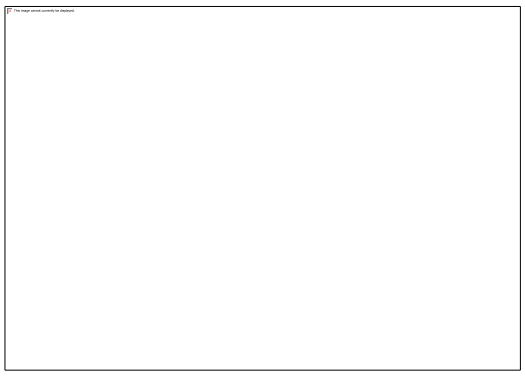
HASI 200 mg/kg on 28^{th} day



HASI 400 mg/kg on 0^{th} day



HASI 400 mg/kg on 28^{th} day



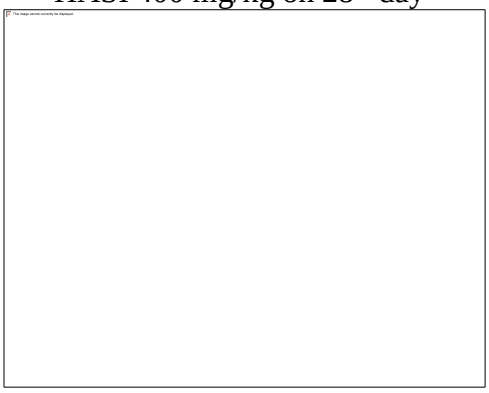


Table 10: Effect of *Spongomorpha Indica L* on Tissue weight & Hydroxyproline content in dexamethasone induceddelayed wound healing dead space model on 28th day

Treatment Group	Tissue dry weight ingm	Hydroxyproline in µg/g of tissue
Dexamethasone 10mg/kg/sc	15.27±4.29	1254.59±1.66
Dexa +GLB+Mupirocin	50.74±2.37***[a]	2158.23±2.89***[a]
HASI 200 mg/kg	39.45±2.97**[b]	1672.62±2.36**[b]
HASI 400 mg/kg	48.53±2.88***[a]	1987.46±2.95***[a]

Values are Mean ± SEM from 6 animals in each group. Significant difference at **p<0.01 & ***p<0.001 when treated groups compared to dexamethasone control. Means bearing same superscript do not differ significantly between treated groups. Means bearing different superscript differ significantly

Figure 14: Effect of *Spongomorpha Indica L* on Tissue weight

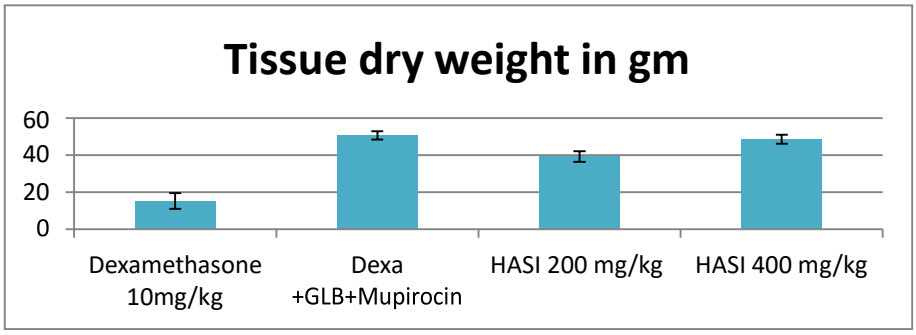


Figure 15: Effect of *Spongomorpha Indica L* on Hydroxyproline in µg/g of tissue

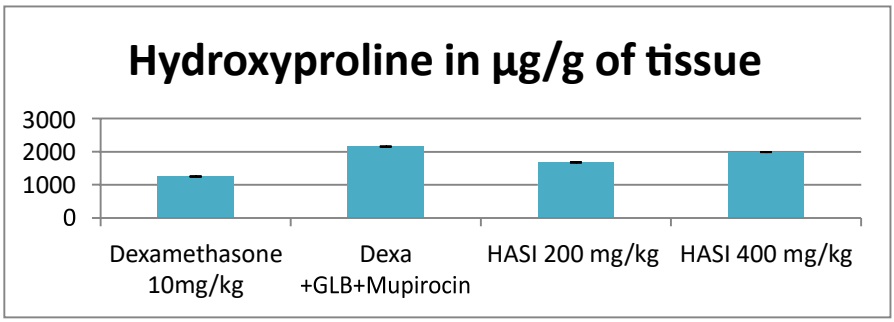
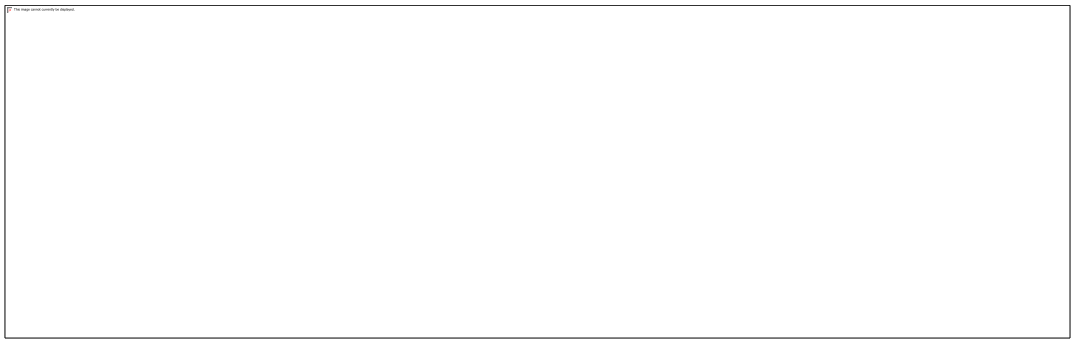
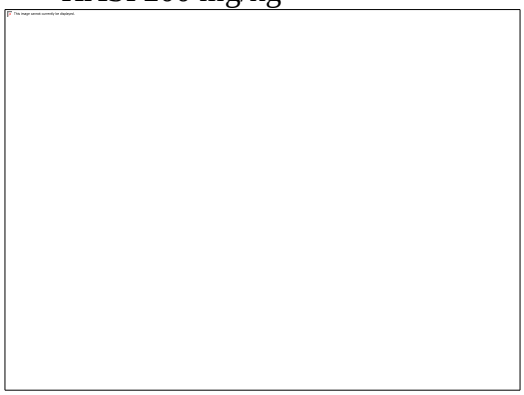


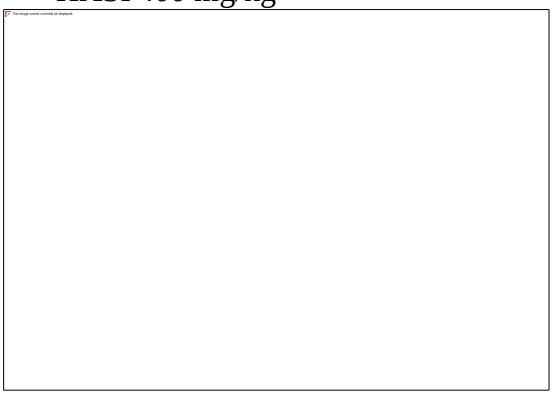
Figure 16: Effect of *Spongomorpha Indica L* in Histopathological Slides of granulation tissue in dexamethasone induced delayed wound healing dead space model

Dexamethasone (10mg/kg/sc) Glibenclamide 5mg/kg + mupirocin ointment



HASI 200 mg/kg HASI 400 mg/kg





Discussion:

Any medication derived from a natural source must have its efficacy and safety assessed and validated by evaluating its active ingredients before it can be accepted into mainstream medicine. The kind of solvent used has a significant impact on how well phenolic, tannin, and flavonoid components may be extracted from plant material. Compared to other solvents, ethanol and hydro-alcoholic extract have the highest concentration of phenolic, flavonoids, and tannin, which makes it a very effective and selective solvent for extracting a large number of bioactive phenolic compounds.[16] It has been discovered that collagen fibers treated with catechin, a plant flavonoid, are stable. Hydrophobic interactions and hydrogen bonding have been demonstrated to be involved in this stabilizing effect.[17]

High exposure to glucocorticoids impairs insulin sensitivity, contributing to the generation of metabolic syndrome including insulin resistance and hypertension.[18] The mechanism by which dexamethasone induces peripheral insulin resistance is by inhibiting GLUT-4 translocation.[16] The underlying mechanism involves increased α_2- adrenoceptor signaling.[20] increased Potassium channel activity[19] and impaired glucose metabolism.[18,19] Although reduced insulin secretion during glucocorticoid treatment can be overcome by blocking adrenoceptor signaling or by inhibition of potassium channel, compelling evidence suggests that the proper functioning of β-cells also depends on cell survival.[20] Accordingly, a reduction of β-cell mass in long-standing glucocorticoid therapy may contribute to the consecutive development of steroid diabetes. The decreased blood glucose level was observed with HASI extract in Streptozotocin and dexamethasone induced diabetic models

Tannins are widely understood to be high molecular weight polyphenol chemicals that exist naturally and combine with proteins to create complexes. Animals rely on tannins as a major source of protein, but the levels of these compounds fluctuate greatly and are

mostly unpredictable. Tannins can also be poisonous to animals and even cause death.[21] When a significant amount of the food contains high concentrations of tannins, the toxic or antinutritional effects typically manifest during periods of stress. The best strategy to battle these tissue damage, unwanted alterations, and health hazards is to consume foods that naturally contain antioxidant activity.[22] Tannins are phenolic chemicals that are commonly employed as astringents in herbal remedies intended to treat wounds. This astringent characteristic causes the wound to shrink and speeds up the rate at which the epithelium forms during the granulation formation and scar remolding stages.[23] Tannins can interact with enzymes produced by microorganisms, including fungi and bacteria, to reduce their activity.[24]

There are many stages of wound healing. entails an acute phase of inflammation at first, which is followed by the creation of collagen and other extracellular macromolecules, which are then excised to leave a scar. Substances that affect one phase might not always affect another.

As a result, our study has employed a variety of models to evaluate the impact of several stages. According to the current research, spongomorpha indica hydroalcoholic extract worked well in every wound healing activity model. Increased wound contraction, decreased epithelization and scar area on the excision model (table 1 and figure 1–5), and a significant increase in skin breaking strength on the incision model in normal rats are the results of the current study using macroalgae extract. These findings are indicative of increased collagen levels due to increased collagen fiber cross-linking. Moreover, a rise in the weight of the dry granulation tissue suggested a larger concentration of protein (hydroxyproline).[25] The findings of the hydroxyproline estimate in Spongomorpha indica supported the aforementioned claim. Previous research has demonstrated that flavonoids' anti-inflammatory, antifungal, antioxidant, and wound-healing characteristics make them useful in medicine.[20-23] Furthermore, it is well known that flavonoids and their derivatives reduce lipid peroxidation by enhancing vascularity and halting or delaying the development of cell necrosis. Due to their astringent, antibacterial, and antioxidant qualities, flavonoids and tannins are also known to promote wound healing processes. These qualities are thought to be the cause of wound contraction and an increased rate of epithelization.[24]

It is commonly recognized that immune cells, such as neutrophils, monocytes, macrophages, and lymphocytes, are engaged in the inflammatory phase of wound healing and that they regulate cytokines, which is essential for the proliferative phase of wound healing. The primary cause of infection and a reduction in the rate at which wounds heal is immunosuppression brought on by diabetes. Therefore, it is imperative to design the agent that is utilized to treat diabetic wounds. Because dexamethasone suppresses the immune system and causes steroid diabetes, the dexamethasone paradigm is the best one for understanding delayed wound healing. According to previous research, polyphenols, flavonoids, and tannins speed up the healing of wounds.[25] It has been established that proanthocyanidins and other tannins are known to promote wound healing.[26-28] Proanthocyanidins, also known as condensed tannins, are a class of physiologically active polyphenol bioflavonoids that are generated by several plants. The current study's phytochemical screening verified the presence of flavonoids, tannins, and phenolic components, among other things, in the spongomorpha indica (HASI) extract. The interplay between the combination of these primary phytoconstituents and different stages of wound healing may account for the HASI extracts' capacity to heal wounds. Thus, the ability of the algae Spongomorpha indica to promote wound healing in rats with delayed wound healing caused by dexamethasone has been investigated. According to our findings, using various HASI extracts at 200 and 400 mg/kg significantly increases wound contraction, breaking

strength, and shortens the epithelization period. In the end, free hydroxyproline and its peptides are released by collagen, the main protein of the extracellular matrix. As a result, hydroxyproline measurement serves as an indicator for collagen turnover.[29] A higher concentration of hydroxyproline signifies a higher production of collagen, which in turn leads to improved wound healing. Thus, both extract doses once again demonstrated the higher Hydroxyproline content's ability to promote quick wound healing. Previous research has shown that the inclusion of tannins, flavonoids, and phenols in HASI may speed up wound healing by either raising the growth factor involved in wound healing or lowering the glucose level, or both. Thus, they might prevent or reverse the stiff blood vessels, which would reduce blood flow to the wound environment.[30] Enough blood flow to the wound site will offer all the nutrients required for the wound cell to be completely replaced by a new epithelial cell. The findings of the dexamethasone model showed a noteworthy variation in the pace of healing among the group treated with HASI extract. It could be caused by the amount of active ingredients in various HASI extract doses.[31,32]

Conclusion: -

The results of this study on Spongomorpha indica hydroalcoholic extract of *Spongomorpha indica* a macroalgae has potential effects in lowering the glucose levels in both streptozocin as well as dexamethazone induced diabetic rats and the effects were found to be more effective than glibenclamide at the highest doses i.e., 400mg/kg. Further studies are in progress at molecular level to explicitly explain more about the mechanism of the antidiabetic activity and compounds responsible for its effect that it may be helpful in the treatment of diabetes that is linked to delayed wound healing. The algal extract reversed the effects of dexamethasone-induced wound healing delay and boosted collagen production, which is involved in the proliferative phase of wound healing. Thus, we deduced that HASI is more beneficial in treating chronic wounds or wounds in patients with immune suppression and diabetes because it accelerates the inflammatory and proliferative stages of wound healing. The HASI extract's effects were dose-dependent; 400 mg/kg proved to be somewhat more beneficial than 200 mg/kg. Its employment in the traditional medical system is justified by its shown ability to heal wounds. More study should be focused on accomplishing this aim as there were not many clinical trials conducted to conclusively establish the therapeutic potential of the discovered natural wound healing chemicals.

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