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COMPARATIVE STUDY OF WOUND HEALING ACTIVITY OF *KHADIRA* (*ACACIA CATECHU* WILLD) AND *ARJUNA* (*TERMINALIA ARJUNA* ROXB.) IN STREPTOZOTOCIN INDUCED DIABETIC WOUND IN SPRAGUE DAWLEY SPECIES ALBINO RATS."

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ABSTRACT

In Ayurveda *Khadira* and *Arjuna* are mentioned in *Kashayaskandha* (Group of astringents). Both possess *Sheet Veerya*, *Katu vipaka* and *Ruksha guna*, *Pramehagna* (anti- diabetic), *Shothhar* (antiinflammatory) properties. The present study was performed to compare diabetic wound healing activity of *Khadira* (Acacia catechu willd.) and *Arjuna* (Terminalia Arjuna Roxb.) in streptozotocin induced albino rats. All rats were injected with STZ intraperitoneally to induce diabetes. After a period of 1 month these diabetic animals were distributed in the different groups (n=6) such as DC (Disease control), STD (Standard drug), Test 1 drug (Khadira), Test 2 drug (Arjuna). Circular excision was made on a dorsum of every animal. Test drugs were applied to respective groups and wound area was measured on days 0, 3rd, 6th, 9th, 12th and 15th for all groups. Aqueous extract of *Khadira* and *Arjuna* both responded both of them have showed diabetic wound healing effect. The wound healing activity is more effective in *Arjuna* extract as compared to *Khadira* extract due to presence of tannin in high intense in *Arjuna* than *Khadira*.

KEYWORD: *Khadira, Arjuna,* Tannin, diabetic wound.

INTRODUCTION

Diabetic wound is the complication of diabetes. Approximately 15% of diabetics will develop a foot ulcer at some point.[1] *Ayurveda* described '*Kleda'* (metabolic waste) & *Bahudravashleshma* (liquefied & excessive amount of *kapha*) as the main causative factors leading to *Dhatushaithilya* in pathogenesis of *Prameha*. In *charka Samhita*, *vimansthan* chapter 8 *Khadira* (*Acacia catechu* Willd) & *Arjuna* (*Terminalia arjuna* Roxb) are included in *kashay skandha*.[2] *Kashay rasa* plays an important role in *kledashoshan* (Absorbing *Kleda*). *Dhatushaithilyanashan* (destroys *Dhatushaithilyanashan*) beside this *kashay rasa* has *vranropan* (wound healing) property.[3] According to *Acharya Sushruta* says that *kushdha* (Skin disease), *vish* (Poison), *shosh* (Tubrculosis), *Madhumeh* (Diabetes) are responsible factor for *Vrana ropan*. *Madhumeh* is a *Krucchasadhya* disease.[4] *Ayurveda* described '*Kleda'* (metabolic waste) & *Bahudravashleshma* (liquefied & excessive amount of *kapha*) as the main causative factors leading to *Dhatushaithilya* in pathogenesis of *Prameha*. In *charka Samhita*, *vimansthan* chapter 8 *Khadira*

(*Acacia catechu* Willd) & *Arjuna* (*Terminalia arjuna* Roxb) are included in *kashay skandha*.[3] *Kashay rasa* plays on important role in *kledashoshan* (Absorbing *Kleda*). *Dhatushaithilyanashan* (destroys *Dhatushaithilyanashan*) beside this *kashay rasa* has *vranropan* (wound healing) property.[3] According to *Acharya Sushruta* says that *kushdha* (Skin disease), *vish* (Poison), *shosh* (Tubrculosis), *Madhumeh* (Diabetes) are responsible factor for *Vrana ropan*. *Madhumeh* is a *Krucchasadhya* disease.[4] Laboratory trials of *Khadira* and *Arjuna* extracts have shown the wound healing property without harming healthy cells. Therefore, there is a continuing search for better control & preventive methods in order to reduce wound healing & related side effects. The over use of synthetic drugs resulting in higher incidence of adverse drug reaction has motivated mankind to go back to nature for safer remedies.

Bhavaprakash Nighantu, *Madanpal Nigantu*, and *Kaiyadev Nighantu* have stated the wound healing and anti-diabetic property of *Khadira*.[5,6,7] The anti-diabetic & wound healing potential of *Arjuna* has been mentioned in *Bhavaprakash Nighantu* and *Kaiyadev Nighantu.*[8,9]

These both drugs possess predominant *kashay rasa*, *sheet veerya* and *katu vipak*.[10] The useful part of *Khadira* as well as *Arjuna* is similar that is bark. *Acacia catechu* Willd is a species of mimosaceae family and *Terminalia arjuna* Roxb is the species of Combretaceae family. It is used in the treatment of some major diseases. The anti-diabetic and wound healing activity are associated with their component like tannin, flavanoids, and alkaloid.[11] Various preclinical investigation has been carried on *Khadira* (*Acacia catechu* Willd) and *Arjuna* (*Terminalia arjuna* Roxb) such pharmacological activities are Anti ulcer activity, Anti carcinogenic and anti lipid per oxidative, Anti microbial, Ant-inflammatory and analgesic, wound healing these activites present in both drugs the plant is enrich with reported wide range of chemical constitute.

The validation of their therapeutic attributes has to be made evidence based by using modern scientific methods. No drug can be tested for its pharmacological action unless it is standardized for its quality and efficacy, identity, purity & quality of raw drugs are the essential requirements for preparation of the drug formulation significance the standard of the drug should be undertaken as initial part of this study. Recently world is being attracted towards medicinal plants but there is question about purity of herbal drugs. Potency and efficacy is closely related with chemical and physical property. So there is need of standardization of medicinal plants. Therefore, for the purpose of quality and purity of drugs standardization of *Khadira* and *Arjuna* has been undertaken [11]. Though *Ayurveda* is a fully developed medical science, but the drugs used in it are not studied on the basis of modern parameters. Considering this fact in mind, throw light in terms of Pharmacognostical and phytochemical analysis of *Khadira* and *Arjuna* was also taken under consideration. It is essential to carry out preclinical studies using animal model. As no research work is available on wound healing activites of *Khadira* and *Arjuna* in diabetic rats. In this study Sprague Dawley albino rats selected for the experiment.It is in vivo study or animal experimentation, animal research and in vivo testing is use of non human animals in experiments that seek to control the variables that affect the behavior or biological system under study. In vivo study is the study of the biological effects of a drug in living organisms. In vivo models or animal models of human diseases are used to observe the physiological effects of a drug. Testing drugs candidates in animals is necessary step to understand the candidate behavior in the whole organism, starting from bioavailability and going through pharmacokinetics, organ exposure, efficacy in animal models of disease, and to a potential toxicity of either the compound itself or its metabolite. These are two most potent drugs widely used in *Ayurveda* in anti-diabetic and wound healing so the efficacy of these two drug in experimental wound healing in the present investigation offer the potential for reaching on understanding of wound healing potencial.

MATERIALS AND METHODS

Plant material
The botanically identified samples, The Bark of *Acacia catechu* willd (*Khadira*) and Bark of *Terminalia arjuna* Roxb (*Arjuna*) were collected. The sample was authenticated for its botanical identity, row drugs and Physico-chemical standardization is carried out in well-known research laboratory Dr. Bhide foundation, SP college in Pune, Maharashtra

Drug standardization
Organoleptically, both drugs have Astringent in taste and Characteristic Odour. Khadira was rough in touch, Greyish brown in Colour while Arjuna was Smooth in touch, whitish in Colour. In microscopic study, it was found that the transverse section of bark of Khadira, showed numerous, uni-to-bi-seriate medullary rays, vessels occurring isolated or in small group of two to four, xylem fibers. And T.S. of Arjuna showed a few outer layers filled with brown coloring matter; phloem Parenchyma and phloem fibers, rosette crystals of calcium oxalate, starch grains, mostly simple to compound.

Physico-chemical standardization
In *Khadira*, Alcohol soluble and water soluble extractive values are 26.17% and 28.63% respectively. Total ash value was 3.22%, Moisture content was 2.67% and pH values was 5.61 whichwas found to be within limit as specified in API. In *Arjuna*, Alcohol soluble and water solubleextractive values are 10.68% and 23.31% respectively. Total ash value was 1.71%, Moisture contentwas 3.02% and pH values was 5.23 which was found to be within limit as specified in API.

Preparation of aqueous extracts and standardization
Aqueous extracts of *Khadira* and *Arjuna* were prepared in APT research center, Pune. For Preparation of aqueous extract of bark of *Khadira* and *Arjuna* Standard method (Soxhlet extraction) wasused. These extracts were used for pre-clinical study. Phytochemical tests revealed the presence oftannin and alkaloids in both drugs extracts. Also HPTLC profile of Aqueous extracts of both drugs tested using different solvents confirmed the presence of Tannin and flavonoids in both *Arjuna* and *Khadira*. In Khadira, Saponin, Triterpinoids, bitter principle were found in high intense than *Arjuna*.

Experimental animals
The experimental protocol was approved by institutional animal ethical committee in APT testing and research private limited, Research project no.35/1819. Rats of strain Sprague Dawley were used for the study. All animals taken were Either Male/Female. Body weight of rats was ranging from 200.0 gm to 250.0 gm. Total animals used for study were 24. The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.

Diabetic wound

After induction of diabetes animals were anaesthetized by injecting intramuscularly ketamine hydrochloride and xylazine in 1:1 concentration. The dorsal fur of the animals was shaved with shaving machine. The area of the wound created was marked on the back of the animals by Marker using circular stencil. Circular excision wound of 300 to 350 mm^2 created to full thickness along the markings using toothed forceps and pointed scissors. Test drugs were applied to respective groups for 15 days. Wound areas were measured on days 0, 3rd, 6th, 9th, 12th and 15th for all groups, using a transparency sheet and a permanent marker and measured by using graph paper and entire wound was kept open. At the end of study Hematology, Biochemistry (Glucose, Total Protein, Urea, Creatinine, Triglyceride, HDL- Cholesterol, Cholesterol, SGPT, SGOT and ALP) was done before sacrifice. Tissues were preserved in 10% formalin for Histopathology of major organs (Skin tissue, Liver, Kidney and Pancreas).

Study design
Table 1: Experimental study design.

Group no.	Group name	Specification (n=6)
1	DC	Disease Control
2	STD	Standard- 0.2% Silver nitrate Gel
3	TEST 1	Khadira Kalk
4	TEST 2	Arjuna Kalk

Administration of drug

Dosing of Standard and test drug was started after confirming induction of diabetes and formation of diabetic wound in animals.

Day on which first dose given was considered as 0 day.

Standard drug**-**After induction of diabetes and formation of diabetic wound; Standard drug was administered for the duration of 15 (0th, 3rd, 6th, 9th, 12th, 15th) days to rats in standard drug group. Silver nitrate was given as a standard drug at the dose of 0.2% locally.

Test drug-After induction of diabetes then create diabetic wound; test drugs were administered for the duration of 15 days to evaluate its anti-wound healing activity.

The gel is prepared which is used for the local application.

The animals were dosed using a stainless steel intubation needle fitted onto a suitably graduated syringe.

Testing parameters
Wound healing
a. Measurement of wound contraction period-

In the wound model, wound area was measured by tracing the wound with the help of transparent sheet using millimeter based graph paper on days 0, 3, 6,9,12 and 15 for all groups.

Wound contraction was measured every 3rd day until complete wound contraction was calculated taking the initial size of the wound as 100% using the following formula:

%wound contraction=

$$\frac{\text{Initial wound size-specific day wound size}}{\text{Initial wound size}} \times 100$$

b. Epithelization period-was calculated as the number of days required for falling off the dead tissue remnants of the wound without any residual raw wound.[181]

C. Body weight- Animals in all groups were weighted on weighting scale weekly and observations were noted.

d. Blood glucose level- 0.5 ml of blood was collected for testing blood glucose level of each animal. It was done by sterilizing the tail of animal with 10% alcohol and then nipping the tail. Test was performed weekly and observations were noted.

Sacrifice of animals

After 15 days of dosing, all animals of four groups were euthanatized by using CO_2 chamber.
Each dead animal was dissected and their organs like Adrenals, Heart, Kidney, Liver, Spleen, Lungs, Gonads, Brain and Pancreas were weighted using weighting scale.

Some tissues were preserved in 10% formalin for Histopathological tests.

Histopathology of major organs like skin, Liver, Kidney and Pancreas was performed of two animals of each group.

Histopathalogical study

At the end of the study, all the animals were anesthetized using ketamine and specimens of wound tissue were collected and preserved in glass vials containing 10% formalin solutions for histological examination. Sections for histological examination. Sections of wound tissue specimens (about 5um thickness) were prepared by microtomy and stained with hematoxylin and eosin (H & E) dye for histological examination.

Statistical method used

For analysis of data obtained from in-vivo evaluation of aqueous extract of *Acacia catechu* Willd and *Terminalia arjuna* Roxb was calculated by using one way ANOVA

test. Total four groups are used in present study. So that, for more than two sample means ANOVA test was used.It is a parametric test. In this test instead of analysing SD, analysis of the variance of more than two means was performed and hence it called 'analysis of variance'. This test is used to test the homogeneity of more than two samples.

OBSERVATION AND RESULTS
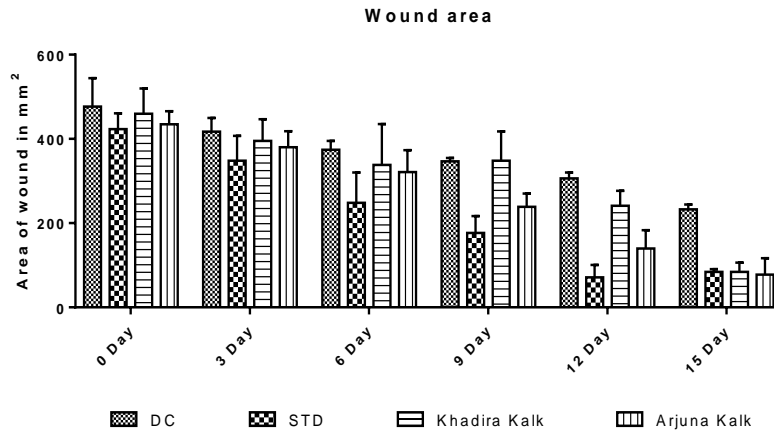
Graph 1: Wound Area (mm^2).

In all animals of 4 groups wound area was measured on 0th, 3rd, 6th, 9th, 12th, 15th day and the readings are as follows.

- In rats of 1st disease control group wound area decreased day by day. Reading was recorded at 6 respective days. The wound area of all rats at 0th day was 476.8 mm^2 and at the end of study that is at 15th day was 232.6.
- In 2nd group i.e standard disease control group mean wound area at 0th day was 423.0 mm^2 and at 15th day was 84.4 mm^2. At 0th, 3rd, 6th, 9th, 12th, 15th day total 6 rats died.
- In 3rd group of diabetic wound albino rats treated with first test drug aqueous extract of *Acacia*

catechu Willd mean wound area at 0th day was 459.7 mm^2 and at 15th day was 84.0 mm^2.
- In 4th group with test drug *Terminalia arjuna* Roxb mean wound area at 0th day was 434.8 mm^2 and at 15th day was 77.6 mm^2. Two rats from this group get died at 12th and 15th day.

There was statistical change observed in the Wound area was found to be decreased on day 6 (STD $p<0.001$; Test 1 $p<0.05$), day 9 (STD $p<0.001$; Test 1 $p<0.01$; Test 2 $p<0.001$), day 12 (STD $p<0.001$; Test 1 $p<0.001$; Test 2 $p<0.001$) and day 15 (STD $p<0.001$; Test 1 $p<0.001$; Test 2 $p<0.001$) in respective treatment group in comparison with DC group.
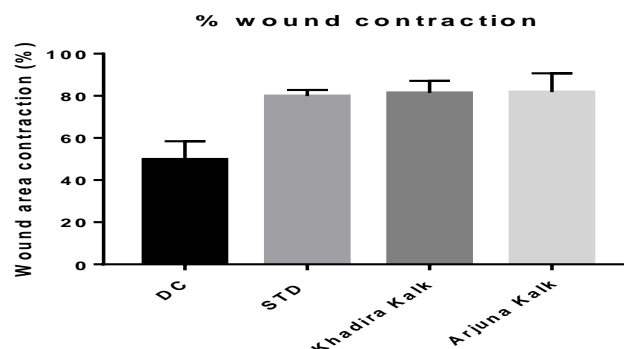
Graph 2: Wound contraction %.

In all animals of 4 groups wound contraction % was measured on 0th, 15th day and the readings are as follows.

- In rats of 1st disease control group wound contraction % decreased day by day. Reading was recorded at 2 respective days. The wound contraction % of all rats at 0th day was 476.8 mm^2

and at the end of study that is at 15th day was 232.6. At 0th, 15th day total 2 rats died.
- In 2nd group i.e standard disease control group mean wound contraction % at 0th day was. 423.0 mm^2 and at 15th day was 84.4 mm^2. At 0th, 15th day total 2 rats died.

- In 3rd group of diabetic wound albino rats treated with first test drug aqueous extract of *Acacia catechu* Willd mean wound contraction % at 0th day was 459.7 mm^2 and at 15th day was 84.0 mm^2. At 15th day 2 rats died.
- In 4th group with test drug *Terminalia arjuna* Roxb mean wound contraction % at 0th day was 434.8 mm^2 and at 15th day was 77.6 mm^2. Two rats from this group get died at 12th and 15th day. At 15th day 1 rats died

Percentage wound contraction was measured at the end of experiment. The percentage of wound contraction was more in all treatment groups in comparison with DC group ($p < 0.001$).

Percentage of Wound Contraction in various Extract
Table 2: Percentage of Wound Contraction in various Extract.

Extract	Day 0th	Day 15th	%Wound contraction
Disease Control	476.8%	232.6%	49.77%
Standard- 0.2% Silver nitrate Gel	423.0%	84.4%	79.87%
Aqueous extract *Khadira* (*Acacia catechu* Willd)	459.7%	84.0%	81.39%
Aqueous extract Of *Arjuna* (*Terminalia arjuna* Roxb)	434.8%	77.6%	81.77%

Epithelialization Period
- Epithelialization in the wound study was very slow in the disease control rats as scab formation was delayed and eschar was not seen to be present till 15th day of the study period.
- However in the standard drug treated rats, scab formation was early and eshar fell on the 9th to 12th Day of wound formation.

- Similarly, in both the test groups the epithelialization was seen early as a sign of collagen turnover and eschar was peeled off much earlier as compared to other groups leading to early wound healing.

Epithelization period
Table 3: Epithelization period.

Extract	Epithelization period
Disease Control	No epithelialization
Standard- 0.2% Silver nitrate Gel	11.40
Khadira Kalk(*Acacia catechu* Willd)	11.25
Arjuna Kalk (*Terminalia arjuna* Roxb)	9.80
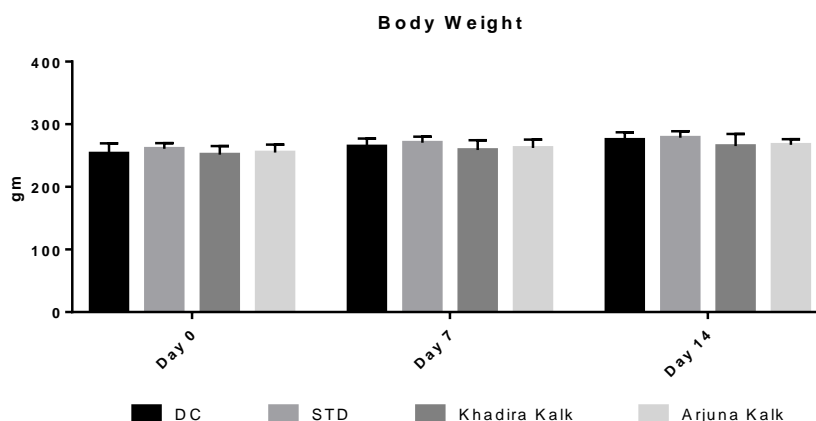
Graph 3: Body weight.

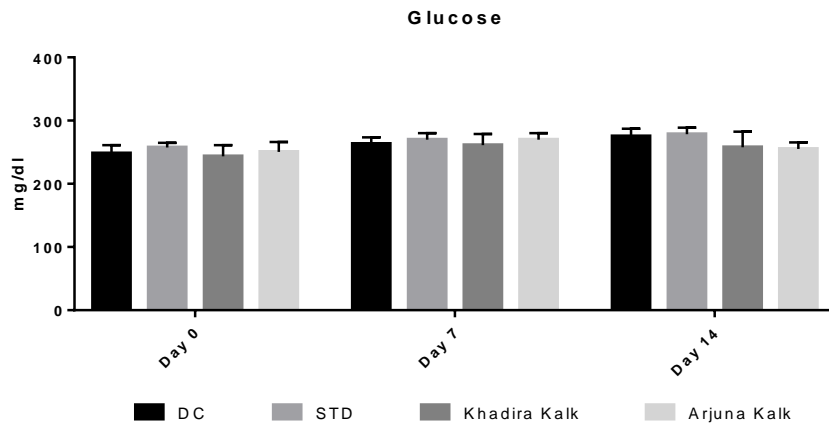In all animals of 4 groups Body weight was measured on 0th, 7th and 14th day the readings are as follows.
- In rats of 1st disease control group body weight increased day by day. Reading was recorded at 3 respective days. The body weight of all rats at 0th day was 253.1gm and at the end of study that is at 14th day was 275.0gm.

- In 2nd group i.e standard disease control group mean body weight at 0th day was. 260.8gm and at 14th day was 278.6gm. At 0th, 14th day total 2 rats died.
- In 3rd group of diabetic wound albino rats treated with first test drug aqueous extract of *Acacia catechu* Willd mean body weight at 0th day was 251.6gm and at 14th day was 265.1gm.

- In 4th group with test drug *Terminalia arjuna* Roxb mean body weight at 0th day was 254.7gm and at 14th day was 267.0gm. One rat from this group gets died at 14th day.
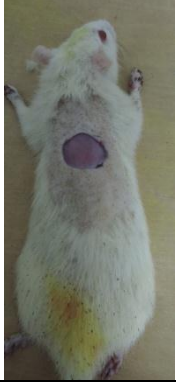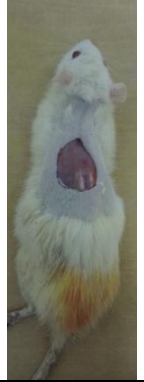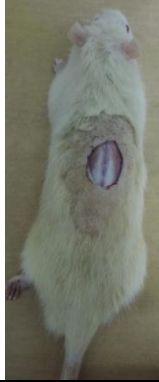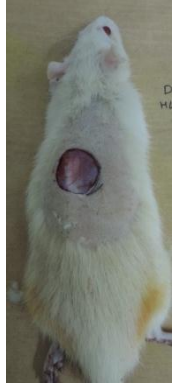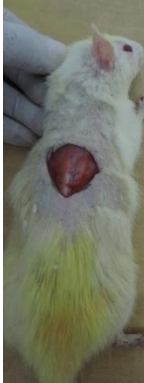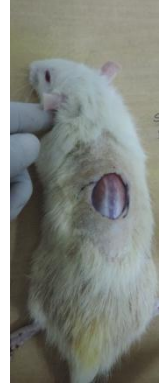

Glucose- Graph 4:
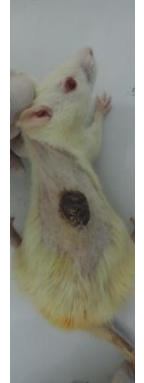
In all animals of 4 groups Glucose was measured on 0th, 7th and 14th day the readings are as follows.
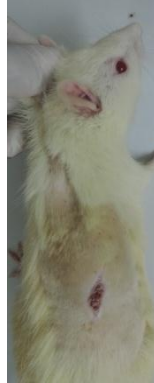
- In rats of 1st disease control group Glucose level at 0th day was 248.0 mg/dl and at the end of study that is at 14th day was 275.0 mg/dl.
- In 2nd group i.e standard disease control group mean glucose level at 0th day was 257.3 mg/dl and at 14th day was 278.6 gm/dl. At 0th, 14th day total 2 rats died.

- In 3rd group of diabetic wound albino rats treated with first test drug aqueous extract of *Acacia catechu* Willd mean glucose level at 0th day was 243.3mg/dl and at 14th day was 257.9mg/dl.
- In 4th group with test drug *Terminalia arjuna* Roxb mean glucose level at 0th day was 250.5mg/dl and at 14th day was 255.0mg/dl. One rat from this group gets died at 14th day.

Khadira Kalk						Dead
	HT	T-RF	LLS	T-HLS	RF	T
Arjuna Kalk						
	HLS	T	T-HLS	LF	W	T-RF

Day 15

DC			Dead		Dead	
	RH	HLS	RF	HT	T	RLS
STD				Dead		
	LH	HLS	T-HLS	T	RF	RLS

	HT	T-RF	LLS	T-HLS	RF	T
Khadira Kalk			Dead		Dead	

	HLS	T	T-HLS	LF	W	T-RF
Arjuna Kalk				Dead		

DISCUSSION
Discussion on analysis of In-Vivo study
Effect on Wound area
Wound area was measured weekly during the study period of 15 days. In disease control group There was statistical change observed in the Wound area was found to be decreased on day 6 (STD p<0.001; Test 1 p<0.05), day 9 (STD p<0.001; Test 1 p<0.01; Test 2 p<0.001), day 12 (STD p<0.001; Test 1 p<0.001; Test 2 p<0.001) and day 15 (STD p<0.001; Test 1 p<0.001; Test 2 p<0.001) in respective treatment group in comparison with DC group.

Effect on % wound contraction
% Wound contraction was measured weekly during the study period of 15 days. Percentage wound contraction was measured at the end of experiment. The percentage of wound contraction was more in all treatment groups in comparison with DC group (p<0.001).

Effect on epithelisation period
Epithelialization in the wound study was very slow in the disease control rats as scab formation was delayed not seen to be present till 15th day of the study period. In the standard drug treated rats, scab formation was early and eshar fell on the 9th to 12th Day of wound formation. , In both the test groups the epithelialization was seen early as compared to other groups leading to early wound healing.

The result indicated a statistically significant reduction in epithelization period of excision wound model compared with contraction (p<0.005).

Effect on Body weight
Body weight was measured during the study period of 15 days. The body weight increased day by day. In disease control group, there was statistically increased in body weight observed on day 15 as compare to day 0.In STD group, Test 1 (*Khadira*) group, Test 2 (*Arjuna*) group showed improvement in body weight on 15th day. The increase in body weight could be due to amelioration of glycemic control and structural protein synthesis.

Effect on glucose (mg/dl) level
After application of an ointment the increase in blood glucose level in disease control (1st group), ST drug (2nd group), *Khadira* extract (3rd group), *Arjuna* extract (4th group) was 27mg/dl, 21.3mg/dl, 14.6 mg/dl and 4.5 mg/dl respectively. This observation indicates that increase in blood sugar level with Arjuna extract was externally less i.e. only 1.8% as compared to *Khadira* extract (6%). Increase in BSL in D.C was 10.89% in ST drug it was 8.27%. Thus in Comparision with all groups, Animals treated with local application an ointment with Aqueous extract of *Arjuna* showed negligible increase in the blood sugar level.

Discussion on Histopathology
The Histopathological examination of rats skin treated with aqueous extract of *Khadira* and *Arjuna* showed increased number of fibroblasts, well-formed hair

follicles with no macrophages and inflammatory cells which are comparable to the standard. Histopathological examination of aqueous extract of Arjuna shows large no of macrophages, inflammatory cells, fibroblasts and well-formed hair follicles than aqueous extract of *Khadira*.

Mode of Action
Probable mode of action of aqueous extract *Khadira* and *Arjuna* as diabetic wound healing agent according to *Ayurveda*

In order for healing of the wound to occur, the wound must undergo *vrana shodhan* (cleansing of wound) and *vrana ropan* (wound healing) passing through the four stages of the wound healing process: *dushta vrana* (septic wound), *Shuddha* vrana (clean wound), *Ruhyamana vrana* (healing wound), and *rooddha vrana* (healed wound) wounds are observed when the doshas are pacified. Both the drug *Khadira* and *Arjuna* shows the presence of *Kashya rasa, Khadira* has *tikta rasa* along with *kashay* rasa.

As *kashay rasa* has *Sandaneeya* property due to its *shleshmaprashaman* (alleviation of *kapha*) & *kledashoshan* (absorption of kleda).[12] According to *Ayurveda Kashay rasa* performs *Raktavishodhan* (Blood purification) & *Kledovishoshan* (absorption of kleda) *karma* (action) helps in wound healing. As kashay ras constricts Rasa & *Raktavaha sira* (blood vessels) in the wound region, *Vranaushth sankocha* (contraction of wound) occurs which helps in speedy wound healing. This rasa has *Shonitasthapna* (Haemostatic) property which stops bleeding in the wound area. Thus all there properties of Kashay Rasa aid fast healing of wound.[13]

Probable mode of action of Aqueous Extract of *Khadira* and *Arjuna* as diabetic wound healing agent according to Modern

The compound identified from the aqueous extract of *Khadira* and *Arjuna* with the help of HPTLC. These compounds have been classified in appropriate chemical groups and data are reported on their pharmacological activites, mechanism of actions which have wound healing effect. Tannin is a main phytoconstituents which are responsible for wound healing activity of *Arjuna*. *Arjuna* shows more wound healing activity than *Khadira.* Activites of these compounds against wound healing are as follows:

Tannins- Tannins are one of the important phytoconstituents responsible for wound healing mainly due to their astringent and antimicrobial property. Tannins are polymeric phenolic substances possessing the astringent property. These compounds are soluble in water, alcohol and acetone and precipitate with proteins. The ability of Tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more. Tannin has been reported to have Anti-viral, antibacterial and antiparasitic effects.[14]

In addition to flavonoids variety of tannins have been isolated from the bark of Terminalia arjuna. Some of the well-known hydrolysable tannins from the bark are pyrocatechols, punicallin, punicalagin, terchebulin, terflavin C, castalagin, casuariin and casuarinin. Some 15 types of tannins and related type of compounds have been isolated from its bark so far. It is also speculated that tannins may be responsible for its astringent, wound healing and anti microbial activity.[15]

Tannin also reduced oxidative stress, ET-1, and inflammatory cytokine levels.

There results strongly document the beneficial effect of aqueous extract of *Arjuna* consisting mainly of Tannins in acceleration of wound healing process. Thus present study validates the claim made w.r.t the plant as well as corroborating the astringent effect of Tannin by drawing the tissue closer together.

Discussion on efficacy of Aqueous extract of *Arjuna* more than *Khadira*

Both drugs contain flavonoids and tannin in high intense. But in *Arjuna* , tannin were found in high intense as compare to *Khadira* i.e *Arjuna* shows more diabetic wound healing effect than *Khadira*. This validates the claim made in *Ayurveda* that *Arjuna* is useful in *Mehavrana* (Diabetic wound) as stated in *Bhavprakash nighantu*.

Thus this investigation conform the use of the gel containing *Arjuna* extract as a wound healing agent as known from *Ayurveda*.

CONCLUSION

Aqueous extract of *Khadira* and *Arjuna* both responded in Comparision with control group drug in animal model Sprague Dawley rats. Both extract more effective, but Arjuna is more effective than Khadira. Based on observation**,** between them aqueous extract of *Arjuna* is more effective as diabetic wound healing agent than aqueous extract of *Khadira* in experimental rats. It is due to *Arjuna* aqueous extract has shown increase in % wound contraction activity as compare to disease control group and as well as *Khadira* aqueous extract. The size of wound in the animals treated with aqueous extract of *Arjuna* was decreased when compared with size of the wound in animals treated with control group and as well as *Khadira* aqueous extract. Based on observation, *Arjuna* aqueous extract shown reduced Epithelization period as compare to disease control group and as well as *Khadira* Aqueous extract. Thus in Comparision with all groups, Animals treated with local application an ointment with Aqueous extract of *Arjuna* showed negligible increase in the blood sugar level. Histopathological examination of aqueous extract of *Arjuna* shows large no of macrophages, inflammatory cells, fibroblasts and well-formed hair follicles than Aqueous extract of *Khadira.* In *Khadira* and *Arjuna,* class of compounds Flavonoids and Tannin were

found in high intensity. The wound healing activity is more effective in *Arjuna* extract as compared to *Khadira* extract due to presence of Tannin in high intense in *Arjuna* than *Khadira*. Pharmacognostical study using *Khadira* and *Arjuna* bark complies the standard monographs, and hence it was concluded that the drug used for the study was *Acacia catechu* willd and *Terminalia arjuna* Roxb. The observed phytochemical results of *Arjuna* and *Khadira* bark are similar to the standard values which are available in A.P.I.These results are stated on the basis of pre-clinical trials and clinical trials are needed. The most imp observation was that no adverse reactions were encountered.

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