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AN EXPERIMENTAL STUDY TO ASSESS THE SYSTEMIC AND DERMAL TOXICITY OF BIODEGRADABLE DERMAL PATCH FOR WOUND HEALING

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ABSTRACT

Background: A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular anatomic or functional continuity of living tissue. Several indigenous drugs have been mentioned for the beneficial effect of wound healing activity in ancient Ayurvedic classics, especially Sushruta Samhita. Group of barks of five trees as Vata (*Ficus bengalensis* Linn.), Udumbara (*Ficus glomerata Roxb.*), Ashwatha (*Ficus religiosa* Linn.), Plaksha (*Ficus lacor* Buch-Ham.) & Parishpippal are together known as "*Panchavalkala*" and mentioned in all Ayurvedic classics. Panchvalkal is reported in different Ayurvedic classics to be used for wound healing. Parishpippal as described in Nighantu granthas,is interpreted by Dalhan as Gardbhand and it is a controversial drug. So for the present Toxic study above four drug have been selected containing Aqueous Extract of four Ficus species i.e Compound Ayurvedic drug(CAD). **Objective**: The objective of this study is to evaluate the Systemic and Dermal toxicity of Compound Ayurvedic drug(CAD) impregnated in Poly (lactic acid) (PLA-CAD) polymer according to the Organisation for Economic Co-operation

and Development (OECD) 404 guidelines. **Materials and methods**: Wister Albino Rats were used for Excisional wound model and divided in to two groups. In Group I, according to OECD 404 guidelines, the test substance i.e., Compound Ayurvedic drug was impregnated in Poly (Lactic Acid) (PLA-CAD) polymer and applied in small area (approximately 1 cm^2) of skin. Bio degradable patch was attached to the skin in such a manner that there should be a good contact and uniform distribution of the patch on the skin. In Group II, the untreated skin areas of the test animal served as the control and dressing was done with only Normal Saline. **Results**: No mortality, abnormal behavior and body weight differences were found after 2 weeks of PLA-CAD bio degradable patch dermal exposure in Group I. No obvious dermal toxicity was observed to PLA-CAD bio degradable patch during the entire 14-day Dermal exposure. No toxicological changes were seen on 14^{th} day in liver, spleen and kidney in histopathological examination. **Conclusions**: These findings suggested that the compound Ayurvedic drug(CAD) impregnated in poly (lactic acid) (PLA-CAD) polymer had no Systemic and Dermal toxic effect as observed in excisional wound model of Wister Albino Rat.This justifies that the compound ayurvedic drug is safe if applied on different kinds of wound.

KEYWORDS: dermal patch, excisional wound model, OCED guidelines, toxicity, Poly (lactic acid).

INTRODUCTION

In Ayurveda, Acharya Sushruta mentioned various drugs and preparations for the management of Chronic non healing ulcer .Out of all these drugs some have Shodhan (wound debridement/wound cleansing) properties and some other have Ropana (wound healing) properties.[1] It is nearly impossible to provide complete management by using single drug. So it is required to use a poly herbal preparation for the management of infective wound, which have both wound cleansing and wound healing properties.[2] Compound ayurvedic drug (CAD) has been described for its beneficial effects on wound management in Ayurvedic Classics and is being used widely for wound healing. The Compound Ayurvedic drugs consists aqueous extract of stem barks of 4 Ficus species i.e Vata (*Ficus bengalensis* Linn.),Ashwatha (*Ficus religiosa* Linn.),Udumbara (*Ficus glomerata* Roxb.),Plaksha (*Ficus lacor* Buch-Ham.).[3]

The dermal patch technology has proven to be fastest, easiest, safest and most economical way to help wound healing.[4] The use of biodegradable polymers in wound management has

been brought into prominence with new innovations in drug delivery systems through this study. Thus with a new dimension for the use of polymeric materials in or as drug delivery devices involves incorporation of biodegradability into the drug delivery system.[5] However, a number of degradable polymers are potentially useful for this purpose including a variety of synthetic and natural substances. Among all these, Poly (lactic acid) (PLA) is the most readily biodegradable polymers. Biodegradation is a natural process by which organic chemicals in the environment are converted to simpler compounds. Biodegradation can only occur within the biosphere as microorganisms play a central role in the biodegradation process.[6]

The wound healing dermal patch was designed on a biopolymeric membrane material which was also the carrier for the drugs along with suitable additives that includes diluents and binders.[7] The proposed wound healing dermal patch was bi-layer where in the first layer supported moisture balance and second layer was for controlling wound infection and cell proliferation. Poly (lactic acid) (PLA) has been used worldwide as bio degradable substrate for nano drug delivery. So in this study Poly (lactic acid) (PLA) was used as a substrate for wound dressing material for impregnation of active ingredients of wound healing drugs.[8]

Objective

The objective of this study is to evaluate the Systemic and Dermal toxicity of Compound Ayurvedic drug impregnated in Poly(lactic acid) [PLA-CAD] polymer according to the OECD 404 guidelines. Histopathological examination of tissue of skin, liver, kidney, and spleen were also investigated after Dermal Patch administration of PLA-CAD. Histopathological findings were assessed after 14-days of post dermal administration, as per Organisation for Economic Co-operation and Development (OECD) 404 guidelines.[9]

MATERIAL AND METHOD

In the present study twelve healthy adult male and female albino rats were taken, housed in poly propylene cages at temperature 20±2°C, with 50–70% relative humidity room in a 12-h light/dark cycle. For feeding them, a conventional laboratory diet with an unrestricted supply of drinking water was provided. After 2 weeks of acclimation, rats were divided in to 2 groups for treatment. Approximately 24 hours before the administration of test substances, fur from rat's body were removed by closely clipping the dorsal area of the trunk of the animals according OECD 404 guidelines.

In GroupI, according to OECD 404 the test substance i.e., Compound Ayurvedic drug impregnated in Poly (Lactic Acid) (PLA-CAD) polymer was first applied in small area (approximately 1 cm^2) of skin. Bio degradable patch was held in place with non-irritating tape. The patch was held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of the exposure period. Bio degradable patch was attached to the skin in such a manner that there should be a good contact and uniform distribution of the patch on the skin. Access by the animal to the patch and ingestion or inhalation of the test substance was prevented. In Group II, untreated skin areas of the test animal served as the Control and dressing was done with only normal saline.

The test site was examined immediately after skin treatment period (1 minute, 1 hour and 4 hours) and then after 60 minutes, 24, 48 and 72 hours and until day 14^{th} of the last administration for signs of erythema and oedema, local toxic effects and clinical signs of toxicity. The dressings were changed every 3^{rd} day till the healing completed.

Dermal reactions were graded according to the OECD instructions. On day14 of post dermal administration, all animals were sacrificed after being anaesthetized and skin, liver, spleen, and kidney under the administration site were excised and histopathological tests were performed using standard laboratory procedures. The tissues were embedded in paraffin blocks, then sliced into 5µm in thickness and placed onto glass slides. After Haematoxylin–Eosin (HE) staining, the slides were observed.

Clinical Observation And Histopathological Assessment

Steps of Histopathology

The tissue needed for histopathology was preserved in formalin 10%. To remove the excess formalin the tissue was washed in running water, put in cassettes-into a bucket and run in a hystokinate for 18hrs for dehydration .The tissue was then placed in wax – in an incubator for about 2hrs.It was taken in an instument containing wax and was then left for hardening. Once the wax got hardened, excess wax was cut off and blocks were prepared. The blocks were then finely cut in a microtome, put to a water bath and 2-3 micron sections were put on albumin coated slides and slides were incubated for about 1 hr at 60°.These slides were then processed with H&E Stain.

Method oF H&E Stain

The slides were first treated with Xyline for 10-15 min for dewaxination. To remove excess Xyline, it was then blotted. The slides were then treated with alcohol for 2-5 min. Then kept in running water so that excessive water gets dissolved. The slides were then treated with Haematoxylin stain for 3-5 min which gave the stain a red colour. The slides were then kept in running water until the red colour becomes blue. The slides were then treated with acid (1% acid HCl) for 2-5 min, here the nuclei took the blue stain and cytoplasm red. The slides were then kept under running water for 10 min. Later the slides were diped in Eosin 1% for few seconds. The slides were then dehydrated with graded alcohol- 90, 95, 100% for a few seconds, blotted and then mounted. The slides were then ready to be visualized under a microscope for Histopathology.[10]

RESULTS

There was no mortality, abnormal behavior and obvious body weight differences after 2 weeks of PLA-CAD bio degradable patch dermal exposure in Group I.

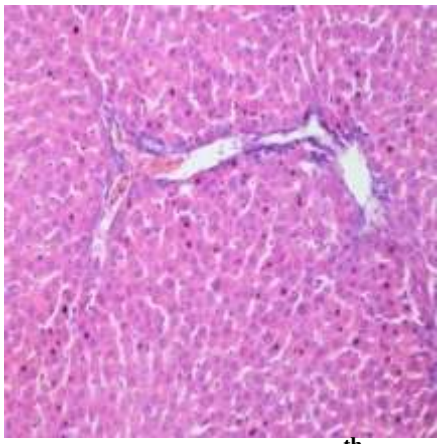
Dermal Toxicity of PLA-CAD Biodegradable Patch

All 6 rats who were treated with drug did not show any symptoms of oedema, erythema and scar formation. No obvious dermal toxicity was observed to PLA-CAD bio degradable patch during the entire 14 day Dermal exposure.

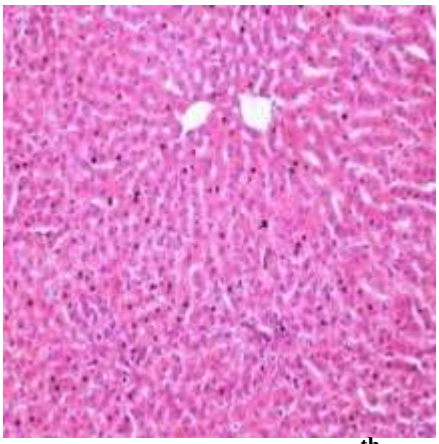
Histopathological Findings of PLA-CAD Biodegradable Patch

On 14day post dermal administration, the pathological observation was performed on skin, liver, spleen, and kidney of exposed albino rats.

Histopathology of Liver at 14th day



Control Group at 14th day

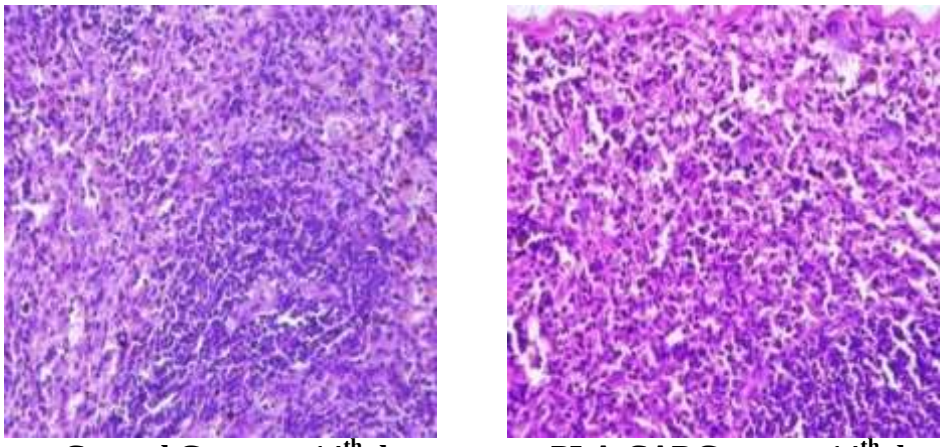


PLA-CAD Group at 14th day

Figure 1

No toxicological changes were seen on 14th day (Figure 1). The histological examination of the liver did not reveal any hepatic necrosis, infiltration in the portal area or liver fatty change on 14th day post dermal administration.

Histopathology of Spleen at 14th day

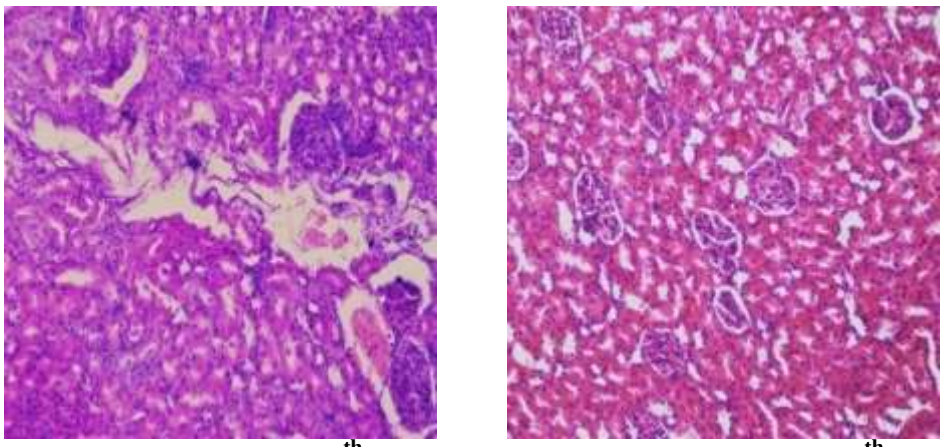


Control Group at 14th day **PLA-CADGroup at 14th day**

Figure 2

No toxicological changes were seen on 14th day (Figure 2).The histological examination of the spleen did not reveal any splenic red pulp congestion, splenic white pulp hyper reactivity or splenic trabecule and sinusoid congestion on 14th day post dermal administration.

Histopathology of Kidney at 14th day

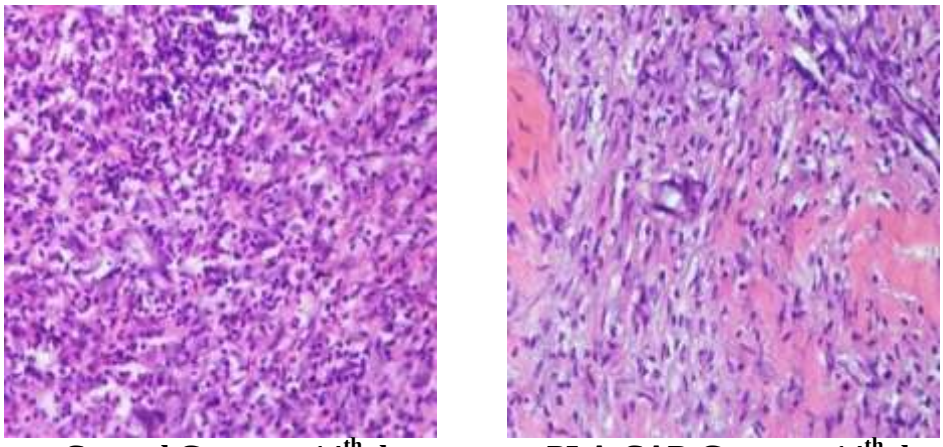


Control Group at 14th day **PLA-CAD Group at 14th day**

Figure 3

No toxicological changes were seen on 14th day (Figure 3).The histological examination of the Kidney did not reveal any congestion or tissue necrosis in malpegian capsule on 14th day post dermal administration of drug.

Histopathology of Granulation tissue at14th day



Control Group at 14th day **PLA-CAD Group at 14th day**

Figure 4

Control Group

More neutrophilic infiltration and necrosed tissue, significant presence of micro abscess, small quantity of healthy granulation tissue, chronic inflammatory cells and appearance of less fibro collagenous tissue (Figure 4).

PLA-CAD group

Less neutrophilic infiltration and necrosed tissue, more quantity of healthy granulation tissue, appearance of more fibro collagenous tissue, less inflammatory cells, marked vascular proliferation in PLA-CAD group (Figure 4).

CONCLUSION

Panchvalkal is the best known Ayurvedic compound in terms of wound healing.In this study among the Panchvalkal 4 of the ficus species were taken. Above result shows that biodegradable patch made from Compound Ayurvedic drug(CAD) impregnated in poly (lactic acid) (PLA-CAD) polymer had no Systemic and Dermal toxic effect as observed in excisional wound model of Albino Rat. This justifies that the compound ayurvedic drug can be the most safe, effective and in expensive treatment for different kinds of wound.

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