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Preliminary investigation of the angiogenic potential of *Ziziphus oenoplia* root ethanolic extract using the chorioallantoic membrane model

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ABSTRACT: The angiogenic potential of an ethanolic extract of *Ziziphus oenoplia* root was evaluated by the chick chorioallantoic membrane (CAM) model (in vitro) in 9 days old fertilized chick eggs. The tested extract was found to increase the number of capillaries on the treated CAM surfaces from three 9 days old fertilized chick eggs. These findings suggested that the ethanolic extract of *Z. oenoplia* root possesses significant angiogenic potential, which may be beneficial in the treatment of wound healing.

KEYWORDS: angiogenesis, chick eggs

INTRODUCTION

Angiogenesis, a complex physiological process required for healing wounds and for restoring blood flow to tissues after injury[1], has become a major focus of study for wound biologists. Wound healing involves different phases including inflammation, granulation, fibrogenesis, neovascularization, wound contraction, and epithelialization[2]. Physiologists have long recognized, however, that neovascularization occurs in normal regenerative processes in wounds. Proliferating capillaries bring oxygen and micronutrients to growing tissues and remove catabolic waste products. This leads to angiogenesis in wounds. Because angiogenesis is required for wound healing, its induction is beneficial in many clinical situations for achieving wound closure. Despite several technological and strategical advances in medicine, wound care has returned to the roots of medicine and embraced some of the natural remedies used millennia ago. There has recently been a surge of interest in the angiogenic roles of natural remedies in healing wounds[3–5].

Ziziphus oenoplia M., belonging to family Rhamnaceae (vernacular name: Siakul) is a shrub, distributed in tropical and subtropical India in dry climates. The roots are astringent, bitter, antihelmintic, digestive, and antiseptic. They are useful for treating hyperacidity, ascaris infection, abdominal pain, and healing of wounds[6]. Although local traditional healers know the wound-healing value of *Z. oenoplia* root, there have been no reports of biological nor pharmacological investigations.

The chorioallantoic membrane (CAM) is a vascular extraembryonic membrane found in eggs of some amniotes, such as birds, and is formed on day 4 of incubation. It is formed by the fusion of the allantois and chorion[7]. Blood capillaries and sinuses form between epithelial cells of the chorionic layer, allowing close contact (within 0.2 μm) with air found in pores of the shell membrane of the egg[8]. CAM from developing chick eggs is routinely used in biological and biomedical research to investigate development[9,10], pathogenesis[11], tumours[12], and to propagate and investigate viruses or helminths[13,14]. The membrane can also be used for testing biomaterials[15]. The CAM model has been used to evaluate the wound-healing potential of natural substances in vitro[16,17]. Therefore, in the present investigation, we made an attempt to evaluate the angiogenic potential for wound healing of the ethanolic extract of *Z. oenoplia* root by the chick CAM model.

MATERIALS AND METHODS

The root of the plant, *Z. oenoplia* was collected from Simlipal Reserve Forest, Mayurbhanj, Orissa during March 2010. A voucher specimen was authenticated by the office of Botanical Survey of India, Howrah, West Bengal and deposited in Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj, Orissa.

Collected *Z. oenoplia* roots were shade dried and then dried in a hot air oven for 30 min at low temperature (37 ± 3 °C). Then dried roots (50 g) were crushed by mechanical cutter and sieved through sieve No. 30. The root crushed and sieved powders were collected and stored in a closed vessel for further use. The residue left after chloroform extraction was dried in an air oven. Dried material (4.2 g) was then placed in a Soxhlet apparatus and extracted successively with ethanol (500 ml). The extraction was continued until completion. The extract was filtered while hot, concentrated, and dried under vacuum at 30 °C. The condensed extract was weighed and kept at 4 °C prior to testing. The percentage yield of *Z. oenoplia* root ethanolic extract was 7.1% w/w.

The ethanolic extract of *Z. oenoplia* roots was subjected to some phytochemical tests to determine the presence of alkaloids (Dragendroff's test), glycosides (Keller-Killiani, Borntrager's, and modified Borntrager's tests), carbohydrates (Fehling's and Molisch's tests), steroids and sterols (Libermann-Burchard test), mucilage (Ruthenium red test), starch (iodine test), tannins (ferric chloride test), proteins and amino acids (Ninhydrin test), tri-terpenoids (tin and thionyl chloride test), saponin (foam test), and flavonoids (NaOH and H_2SO_4 test) [18–20].

The chick CAM model was used as an in vitro model [16,21] to assess the angiogenic activity of *Z. oenoplia* root ethanolic extract. Nine days old fertilized chick eggs were selected and a small window in the egg shells was opened. Then, a sterile disc of methylcellulose loaded with the extract (40 µg/disc) was placed at the junction of two large blood vessels (Fig. 1). The window was resealed with adhesive tape and the eggs were incubated at 37 ± 1 °C in a well-humidified chamber. After 72 h, the tape was opened and new blood vessel formation was observed and compared with the control eggs containing discs without the extract. This test was performed in triplicate.

Fig. 1 Placing of methylcellulose disc with extract on chorioallantoic membrane (CAM).

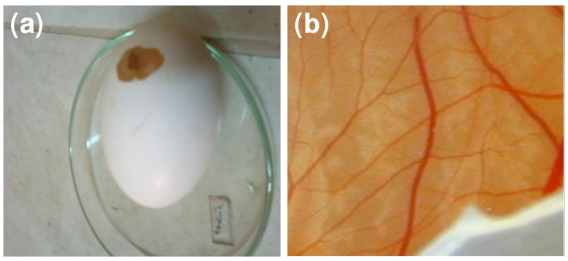

Fig. 2 Chick CAM after the treatment with ethanolic extract of *Z. oenoplia* root: (a) control containing methylcellulose disc without plant extract; (b) treatment containing methylcellulose disc with ethanolic extract of *Z. oenoplia* root (40 µg/disc).

RESULTS AND DISCUSSION

The phytochemical identification tests carried out on *Z. oenoplia* root ethanolic extract confirmed the presence of alkaloids, carbohydrates, starches, mucilages, tannins, saponins, proteins and amino acids, tannins, steroids, and sterols. These tests also confirmed the absence of glycosides, tri-terpenoids and flavonoids.

In the chick CAM model, the ethanolic extract of *Ziziphus oenoplia* M. root showed an increase in density of blood capillaries on the treated membrane surface. This phenomenon indicates good angiogenic activity of *Z. oenoplia* root ethanolic extract at 40 µg/disc dose compared with the control disc (Fig. 2). Angiogenesis is important in normal processes such as the development of the embryo, formation of corpus luteum, and wound healing [16,22]. Again, angiogenesis during wound repair serves the dual function of providing the nutrients demanded by the healing tissues and contributing structural repair through the formation of granulation tissue [16,23].

From phytochemical identification tests, the nature of the compound responsible for angiogenesis present in the tested extract and the possible mechanism responsible for this was not identified. The possible phytoconstituent(s) may be any or a combination of alkaloids, carbohydrates, starches, mucilages, tannins, saponins, proteins and amino acids, tannins, steroids and sterols.

It can be concluded that the ethanolic extract of *Z. oenoplia* root has a positive angiogenic potential which could be beneficial in wound healing. The in vivo angiogenesis as well as wound healing evaluations are a subject for future studies.

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