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Original Article

Python fat: effect on collagen levels of human keloid tissue.

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ABSTRACT

Aim: The present study is aimed at verifying the curative powers of python fat by subjecting it to scientific analysis with a view to showing evidence of its actual effects on keloid tissues, since it has been applied in complementary and alternative medicine (CAM). Method: In this study, the effect of python fat on collagen levels of human keloid tissue culture was determined. Keloid tissues, surgically removed from 9 patients were incubated with python fat. There was subsequent isolation, purification and quantitative determination of collagen levels after incubation of the keloid tissue in a 48hr conditioned media and with increasing doses of python fat. Result: The result gave a mean collagen concentration (mg/ml) of 91.07, 85.78, and 79.95 respectively. The results also reveal a successive decrease in the means of collagen concentration by 7.48%, 12.85% and 18.77% of their estimated original values and a dose-dependent increase in collagenase activity with increasing amount of python fat. Conclusion: The study shows that python fat decreases the collagen concentrations in the keloid tissues by increasing the collagenase activity, and as such can be regarded as a suitable antikeloidal agent for use especially by complementary and alternative medicine (CAM) practitioners.

1. Introduction

Health care is one of the most important issues and major problems pertaining to the quality of life in Africa today. Complementary and Alternative Medicines (CAM), defined as medical and health care systems, practices, and products that are not currently considered an integral part of conventional medicine [1], is still practiced in Africa and in Nigeria, especially in the rural areas. Each particular therapy may be considered complementary if it is used in addition to conventional medical treatment; it is viewed as an alternative if the patient decides to use it in place of a prescribed medical treatment and the source of the primary ingredients used by the alternative medicine practitioners are wild animals and plant species. Python fat is extracted from the wild pythons, with species including Python sebae sebae, Python molurus, Python tigris, etc [2]. It is golden yellow in colour and melts to pale yellow oil on standing. Traditionally it has been used in the treatment of rheumatism, boils, keloids and broken bones [3]. Although there are no established scientific basis for the use of a particular animal or plant material in the treatment of a defined ailment, alternative medicine practice (traditional medicine) continues to be popular due to effectiveness, prohibitive costs of modern medical facilities and the ease with which the practitioners can be reached [4]. Keloids are benign fibrous growths that result from excessive dermal connective tissue that forms in response to trauma in predisposed individuals [5]. Clinically, their appearance is highly variable, reflecting the variation in antecedent trauma. Their location and configuration, but not their size, appear predetermined by the site of skin trauma [6]. Keloid scars are characterized by fibroblastic proliferation and excessive collagen deposition [7, 8]. They develop as a result of an abnormal wound healing that does not usually regress spontaneously, and they tend to recur after excision [9]. The collagen fibers are thickened, glassy, pale-staining and faintly refractile. The predisposition to form keloids is found predominantly in people of African, Asian, and Hispanic descent [10] and although this disfiguring and sometimes disabling disorder of wound healing significantly impairs the quality of life, it is understudied relative to other chronic skin disorders [11].

The general objective of the present study is to verify the curative powers of python fat by subjecting it to scientific analysis with a view to showing evidence of its actual effects on keloid

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tissues. The specific objective is to ascertain the effect of python fat on collagen levels of human keloid tissue culture. Results from the study will not only provide information on the effect of python fat on keloid tissues, but will also provide a scientific basis for its use, considering the fact that Africans are predisposed to form keloids. Also since facilities are now available for scale-up production of desired biomolecules and outright synthesis of its analogues (Rang et al., 1999) administration of the pure python fat, topically, no doubt will be more potent compared to concoctions used by the traditional healers.

2. MATERIAL AND METHODS

Samples of python fats and oils were bought at the traditional medicine area in Ogbete Main Market Enugu and were used for the study. Surgically removed keloid tissues were got from the National Orthopaedic Hospital Enugu, Enugu State. All the chemical and reagents used were of analytical grade (Analar).

Analytical Methods:

Determination of collagen levels of cultured keloid tissues required column chromatographic and absorbance spectrophotometric techniques.

Human Keloid Tissue Culture and Preparation of Culture Supernatant.

Human keloid tissue culture was prepared by the method of Wilson and Walker [14]. Four culture vessels of Trowell's type-II chamber for organ culture were each poured 100ml of suspension of Parker-199 synthetic medium containing different metabolites and foetal calf serum as stimulant to cell growth. 5 g of human keloid tissue were cut in slices of about 1.5mm by means of a microtome and placed on the grid within each culture vessel. The grid is a permeable sheet of Millipore material that provides high surface area for delivery of nutrients to the tissue on solid support. The pH of the medium was maintained at between 7.3 and 7.5 by means of a 30mM bicarbonate buffer. Subsequently 0ml, 5ml, 10ml and 15ml python fat were put into the control, first, second and third culture vessels respectively. The temperature of the incubating chamber was set at 37°C and 95% (v/v) oxygen (Original v 3.4L) in the gas phase infused through an inlet in the innermost cells of the slices. The culture vessels were then put in their positions in Trowell's type-II chamber for organ culture and the tissues therein allowed to incubate for 48hrs. Subsequently, the cultured tissues were minced and put in 25ml of 0.25 mM acetic acid solution. The minced tissues were homogenized at 650rpm and the supernatant collected.

Calibration of Chromatographic Column and Determination of Collagen Levels of Keloid Tissue.

A 300ml capacity chromatographic column was calibrated by pouring 5ml of 10g/L collagen analytical grade in a well-packed Agarose -4B gel. The collagen was subsequently eluted with an isocratic 10mM acetic acid buffer system and fraction number × (FN×) with highest absorbance at 280nm and 260nm determined. The retention volume (VR), the retention time (tR) and thus the rate of flow (F) of the fractions were also determined. Five (5) ml of culture supernatant from the homogenized and centrifuges keloid tissues was poured into Agarose-4B gel column and eluted with an isocratic 10mM acetic acid buffer. The absorbance at 280nm for fraction number × (FN×) for the 32 supernatants including controls and tests were then read off. Collagen concentration in sample is given by:

$$(C)_{mgmL^{-1}} = 1.55A_{280} - 0.76A_{260.}$$

RESULTS

The calibration of chromatographic column gave results as shown in table 1.

Table 2 shows collagen concentrations obtained with increasing dose of python fat in culture and also shows a percentage decrease in collagen concentration, relative to estimated original value.

Table 1. Results on caliberation of chromatographic column

Parameter	Values
V_E	20ml
FN_X	4
V_R	80ml
t_R	6mins
F	$0.22mls^{-1}$
$^4A_{280}$	11.40
$^4A_{260}$	13.25
$^4(C)$	$7.6 mg ml^{-1}$

Where:
V_E = Elution Volume
FN_X = Fraction Number x
V_R = Retention Volume
t_R = Retention Time
F = Flow Rate
$^4A_{280}$ = Absorbance at 280nm of Fraction Number 4.
$^4A_{260}$ = Absorbance at 260nm of Fraction Number 4.
$^4(C)$ mg ml^{-1} = Concentration of collagen in fraction Number 4.

Table 2. Collagen concentration obtained with increasing dose of python fat in culture.

[C] 0 ml (mgml-1)	[C] 5 ml (mgml-1)	[C] 10 ml (mgml-1)	[C] 15 ml (mgml-1)
98.43	91.07	85.78	79.95

DISCUSSION

In vitro studies on the effect of python fat on collagen levels of human keloid tissue culture was necessitated by quantitative connective tissue analysis, which indicated that collagen was the predominant extracellular matrix component in keloids [14]. In the present study, investigations were carried out on 9 patients, 4 males and 5 females between 20 and 50 years who had lesions on the chest, shoulder, ear lobe and neck. Collagen levels in 48hr conditioned media of keloid derived cultures were determined quantitatively using column chromatography and absorption spectrophotometry as in the method of Wilson and Walker, [13]. Our results reveal that collagen studies were significantly lower in cultures exposed to python fat than in the controls. The individual response of keloid tissue to increasing dose of python fat varied slightly and consistently. There was also a gradual decrease in the means of collagen concentration (Table 2), as the dose of python fat increased in culture. A mean collagen concentration of 98.43 mg/ml obtained for 9 keloid tissues incubated without python fat and under test (To), provided a background against which the effect of python fat on keloid tissues can be assessed. Mean collagen concentrations in mg/ml obtained in 5ml, 10ml and 15ml of python fat were 91.07, 85.78 and 79.95 respectively. This corresponds to a decrease by 7.48%, 12.85% and 18.77% of the collagen concentration in control. By implication, 5ml of python fat caused a decrease by 7.48% of the collagen levels of keloid tissue whereas 15ml of the same substance caused a decrease by 18.77%. This indicates a dose dependent increase in collagenase activity with increasing amount of python fat.

CONCLUSION

These results of our study suggest that python fat has a constituent, which decreased collagen accumulation in vitro, possibly with increased collagenase activity. This also shows its ability to serve as a potential anti-keloidal agent, since keloids have been reported to have excessive collagen deposition. This also scientifically supports its use by complementary and alternative medicine practitioners in treatment of keloids.

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