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Research Article

Development and Evaluation of Novel Wound Healing Hydrogels Based on PEGylated *Mucuna flagellipes* Gum

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ABSTRACT

This work was designed to evaluate the drug delivery and therapeutic (wound healing) potentials of PEGylated *Mucuna flagellipes* seed gum. The gum was extracted, PEGylated, dried, pulverized and sieved. The physicochemical properties of unPEGylated and PEGylated gum were determined. The wound healing activity of the PEGylated gum was evaluated using contraction of excision wounds on rat skin treated with varying gum-PEG ratios of 1:1, 1:2, 2:1, 1:3, 3:1, 1:1.5(2:3),1:0 and 0:1, and their effects compared with standard antibiotic (cicatrin powder) and the untreated wounds by monitoring the wound healing process for three weeks. Results obtained from the characterization studies showed that there were enhanced physicochemical and biological properties in the PEGylated gum. The PEGylated gum showed significant increase in wound healing ($p < 0.05$) compared to the unPEGylated gum (negative control) and positive control (cicatrin powder). In all ratios, there was a progressive increase in rate of wound healing and this was due to the contributory action of the combined gum and PEG to wound healing. However, the 1:1.5 ratio PEGylated gum healed the wound faster than cicatrin powder; it healed the wound after 15 days, while cicatrin healed after 18 days. Thus, PEGylated *Mucuna* gum could be used as wound healing agent especially at optimal gum-PEG ratio (1:1.5). This study has shown that PEGylated mucuna gum has values in management of superficial skin wounds.

INTRODUCTION

In normal skin, the epidermis and dermis exist in steady-state equilibrium, forming a protective barrier against the external environment. Wound, a disruption of normal anatomic structure and function of skin and living tissue [1] as a result of physical, chemical, microbiological or immunological injury [2], may be chronic (those caused by pressure, vascular, diabetic ulcer etc) or acute (those caused by surgery, trauma, burns etc) [3–5]. Wound healing or cicastration, a complex process in which the skin or another organ tissue repairs itself after injury [2], occurs by an interconnected process of regeneration of dermal and epidermal tissues that involve the migration, proliferation, adhesion and differentiation of cells [6, 7], and is influenced by local and systemic factors of collagen fibers by reducing neovascularization and epithelialization rate under the regulation of special mediator that secretes blood platelets, macrophage, lymphocyte and so on [8–10]. Once the protective barrier of the skin is broken, the normal physiologic process of wound healing is immediately set in motion [11]. Wound healing starts from the moment of injury and can continue for varying periods of time depending on the extent of wound. In fact, various biological and physiological stages of the healing process of a wound can be summarized into five consecutive cascades of events of haemostasis, inflammation, migration, proliferation and maturation [12–14]. Wound healing process can also be based on three types of principle/intensifying stages- healing by first intention, healing by second intention or healing by third intention [15, 16]. Wound healing is mediated through the phases by a wide range of chemically co-ordinate cellular processes as well as hormonal influences [17,18]. Clinical studies have demonstrated that a measure of the tissue

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microbial load in a wound can predict delayed healing or infection [3].

In ancient times, a suitable material is usually employed to cover the wound in order to prevent any infection for effective wound healing. In modern times, bandages are used to cover the wounds for effective wound healing. For an effective design of a functional wound bandage, characteristics of the wound type, wound healing time, physical, mechanical and chemical properties of the bandage must be taken into consideration [19]. Ultimately, the main purpose is to achieve the highest rate of healing and the best aesthetic repair of the wound [15–18]. Several drugs, mostly antibiotics, have been used in the treatment of various types of wound, and many have also been formulated as ointments, bioadhesives and wound dressings used in the treatment of severe skin wounds or ulcers including bedsores and burn wounds [19–21]. Generally, formulations are selected based on the disease stage and the causes of the wound. Penicillin and streptomycin have been widely employed in combating post-operative infections in man and animals. There has been increasing resistance to some of the orthodox antibiotics used in wound healing, owing to the high level of bacteria resistance that has ravaged the healthcare system as a result of adulteration, poor combination chemotherapy and inactivation of the antibiotic by some enzyme producing bacteria [22]. To circumvent this problem, the use of phytomedicines became imperative, where the wound healing activities of plants, for example, have been explored in folklore [23, 24]. The significant successes recorded have led to investigation into medicinal plants with a view to authenticating the acclaimed properties of the plants. Records have it that different parts of plants used for wound healing contain some active principles or components that are antimicrobial and nutritive in function [25, 26]. Plants and plant products present some hope to scientists, serving as an alternative avenue to drug discovery with a view to finding lasting solution to medical conditions that have not only ravaged mankind but which also have proved very resistance to western drugs. In this regard, formulations of the plants and parts or extracts thereof have been utilized to achieve effective wound healing [27–29].

Mucuna flagellipes, which is popularly known as "ukpo" by the Igbo-speaking people of Southeastern Nigeria, is a legume belonging to the subfamily papilonacea, and it comprises pods covered with brownish dense whisker – like hairs called trichomes that are irritating when they come in contact with the skin or eyes [30]. It is a tropical forest climbing perennial herb and one of the lesser known, neglected and under-utilised legumes of Nigeria [31]. The plant, which occurs naturally in Sierra Leone to Nigeria, Zaire and Uganda, grows up to 12 m long by 3cm diameter at base of riverine and swamp forest, is an annual crop and a climber and can be cultivated more than once a year [30]. It is high yielding; and bears pods which contain usually three to four seeds per pod [32]. Among the natives, the endosperm which is rich in gum is pulverized and used as thickener in many traditional food preparations. The functional properties as well as the anti-nutritional properties of *mucuna flagellipes* have been studied by some authors [33]. In addition, several authors have reported on its suitability for application in processed foods including use as a rheology modifier and stabilizer [34, 35] and film former [36]. The pods (Figure 1) are green/white when immature but black when dry and mature. The seeds are usually dark brown to black in colour. The seeds (Figure 1) can be used either when they are fresh and tender or when dry. The seeds of *Mucuna flagellipes* when mature are processed into flour which can then be used as soup thickener, flavor and stabilizer [31]. Besides being used as soup thickeners, they can be used as additives in other foods to impart desirable textural and functional properties such as enabling fast coagulation in food preparation of the different finished products particularly the "convenience foods" which contain one or more gums [37]. It is among the food thickeners consumed in South Eastern Nigeria [31]. It has been reported that nutritive value of the plant had been improved by heat treatment due to reduction of their anti-nutritional factors content (L-dopa, phenolics, tamin, haemagglutinins, trypsin and chymotrypsin inhibitors, phytic acid, saponins and cyanogenic compounds) by them [38]. Currently, there is a growing awareness on its potential importance as food, pharmaceutical and other commercial importance [39]. Both the seed and the leaf of *M. flagellipes* have high economic, pharmaceutical and domestic uses. The seeds have been reported to be rich in protein, fats, carbohydrate and minerals, and vitamins [30, 32], while the leaf was reported as being used to formulate local hair dye [31]. On dry weight basis, *M. flagellipes* ("ukpo") contains high percentage of proteins (20.4%), carbohydrates (61%) and fat (9.6%). Mucuna gum is a

galactomannan and has D-galactose and D-mannose as the main sugars [40]. The excellent nutritional value of the legume in terms of proximate, mineral composition makes it necessary for it to be used as compliments in African diets which are mainly roots and tuber based [35], and due to the fact that the seeds are rich in protein and carbohydrates, they compare favourably with high protein animal sources such as oyster, beef, pork and marine fishes [34]. The gum has potential for use as binder in ephedrine tablets [39,41]. In herbal medicine, a decoction of this plant is taken to arrest diarrhea and headache; to expel tapeworm; as a uterine stimulant as well as an aphrodisiac and anti-inflammatory agent [30, 42–43].

In spite of the various uses of this plant in food and as medicine, the wound healing activity of Mucuna gum and PEGylated mucuna gum has not been investigated. In view of this, in this work, *Mucuna flagellipes* seed gum, a hydrophyllic natural polysaccharide gum extracted from *M. flagellipes* seed endosperm, was evaluated for its wound healing potential prior to and after PEGylation. Wound healing activities of many plants such as *Ocimum gratissimum* and *Vernonia amygdalina* have been enhanced in our earlier studies by combining them with biopolymers such as mucin and polyethylene glycol via mucination and PEGylation, respectively [22, 44]. PEGylation, the process of covalent attachment of PEG polymer chains to another molecule usually a drug or therapeutic proteins or the molecular attachment of PEGs with different molecular weights to active drug molecules or surface treatment of drug bearing particles with PEGs, is associated with many pharmacokinetic outcomes including increase in overall circulation life span, tissue distribution pattern and elimination pathway of the parent drug/particle [45]. Various grades of polyethylene glycol (PEG), hydrophilic, flexible, non-immunogenic and non-toxic biodegradable macromolecule, have been used in drug delivery; PEG has also been mixed with honey and pollen extract for treating skin lesions in milking cows' and for preventing peritoneal adhesions by promoting non-adherent healing, and it has also been employed by our research team to potentiate the wound healing effect of *Vernonia amygdalina* when formulated as solid dispersions [44].

The objectives of this study were to assess the effects of PEGylation on the physicochemical characteristics of *Mucuna flagellipes* seed gum extract and to assess the activities of the different gum/PEG ratios of the *M. flagellipes* gum compared with the unPEGylated gum and the standard (cicatrin powder) in wound healing using albino rats.

Figure 1: Pods and seeds of Mucuna (cf. M. sloanei) from the Monteverde Cloud Forest of Costa Rica. The pods are covered with dense, whiskerlike hairs (trichomes)

Source: http://waynesword.palomar.edu516 × 206Search by image

MATERIALS AND METHODS

Materials:

Polyethylene glycol (PEG) 6000 (Ph. Eur. Carl Roth GmbH + Co.KG Karlsruhhe Germany), acetone, benzene, diethyl ether, acetic acid, formic acid, carbon tetrachloride and toluene (BDH, England), ethyl acetate, chloroform and methanol (Sigma-Aldrich, Germany), Methylated spirit (Kenol Pharm Ltd, Nsukka, Nigeria), Ketamine® (Laborate Pharmaceutical, India), cicatrin powder (Glaxo Welcome, UK), distilled, deionized and purified water (Lion Water, Nigeria). Seeds of *Mucuna flagellipes* were procured from Nsukka commodity market and properly identified in the Department of Pharmacognosy, University of Nigeria, Nsukka. *Mucuna flagellipes* seed gum was processed in the sterile laboratory of the Department of Pharmaceutics, University of Nigeria, Nsukka. Sprague-Dawley albino rats were obtained from animal house, Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. All other chemicals and reagents were of analytical grade and were used without further purification.

Extraction of *Mucuna flagellipes* seed gum

This was carried out using established procedure with slight modifications [34]. The shells of the *M. flagellipes* seeds were removed and the black seed coats scraped off with knife. The seeds were sun-dried for 3 weeks and then pulverized by means of a hammer mill. Then 593g of the powder was weighed out into a marked plastic bucket, and 4 litres of distilled water containing 1% sodium metabisulphite was introduced to

form primary solution. The 1% sodium metabisulphite prevents oxidative blackening by inhibition of the enzymes. To the primary solution was added additional 6 L of the distilled water containing 1% sodium metabisulphite. The solution was left for 24 h for full hydration and solubilization of the gum. The solution was next pressed using muslin cloth, after which the residue mass remaining in the muslin cloth was discarded. The pressed out slimy solution was treated with acetone (in the ratio of 10 ml of gum to 8 ml of acetone) to precipitate out the gum. The gum was washed several times in a fresh acetone till non-slimy mass was obtained. It was pressed in muslin cloth again and then divided into two for drying. One portion was dried in the oven at 60 ^{o}C and the other portion air-dried. The weight was taken, followed by pulverization into powder with mortar and pestle. The powder was sieved using sieve number 25 to produce the fine powder of the *Mucuna flagellipes* seeds gum. The yield was calculated and it was then stored in amber coloured bottle.

PEGylation of *Mucuna flagellipes* seed gum

PEGylated and unPEGylated *Mucuna flagellipes* seed gum were generated from PEG 6000 and *M. flagellipes* seed gum by a method of PEGylation called controlled coacervation in aqueous medium [45, 46] using gum-PEG ratios of 1:1, 1:2, 2:1, 1:3, 3:1, 1:1.5(2:3),1:0 and 0:1. Briefly, in each ratio, PEG 6000 was dissolved with 100 ml of distilled water in a beaker and the resultant solution used to dissolve the gum. Each ratio mix was put in a container and incubated at 37 ^{o}C for 72 h to allow for gum-PEG interaction followed by evaporation in evaporating dish to concentrate the gum-PEG solution, then oven-drying at 45 ^{o}C for 3 days and cooling under fan for 3 h. The PEGylated gum was further pulverized in a mortar and sieved into fine powder using sieve number 25.

Characterization of the *Mucuna flagellipes* seed gum

Determination of pH

The pH values of the 0.5, 1.5, 2.5, 3.5 and 4.5 (%w/v) aqueous dispersions formed were determined using pocket sized digital pH meter (HANNA Instruments, India). The pH meter was adjusted to neutral with water and then dipped into 15ml of each concentration of the dispersion. The pH value was recorded. The procedure was repeated three times and the average taken.

Determination of particle true density

The true density (ρ_t) was determined using a 50 ml pycnometer with acetone, which is a non-solvent for *Mucuna flagellipes* gum powder, a hydrophilic polymer [34, 43], serving as the displacement fluid. The pycnometer was weighed empty using an electronic balance (Sauter, Germany) and its weight was recorded as W_1. It was then filled with acetone and re-weighed giving a weight, W_2. The difference in weight was recorded as W_3. A 1g quantity of each batch was placed in the empty pycnometer, which was then filled with acetone. This set-up was then covered and placed in a water bath maintained at 40 °C for 5 min to drive off entrapped air. The pycnometer was then weighed after allowing it to cool for 15 min to give a weight, W_4. The true density of each sample was then calculated by using equation 1.

$$\rho_t(g/ml) = \frac{W_2 \, X \, W_3}{50\,(W_1+W_2+W_3-W_4)} \quad \ldots\ldots\ldots \quad (1)$$

Where ρ_t is the true density.

Rheological evaluation (Capillary viscometry)

The Ostwald U-tube viscometers were first rinsed with deionized water and then acetone. They were turned upside down and allowed to dry completely and then clamped upright maintaining the same height and level. The bulb of each viscometer was filled with purified water via the mouth of the wider arm of the viscometer. The water in the bulb was sucked up from the open end of the narrow arm to fill the reservoir bulb and capillary until the water level is above the reservoir bulb. The narrower open arm was closed with finger to hold the liquid in place. The finger was released and the descent of the liquid in the upper portion of the smaller arm watched. The time required for the water level to pass between the two marks (upper and lower calibrated marks) of the reservoir bulb was accurately recorded. After performing the experiment using deionized water, the same procedure was repeated for each of the 0.5, 1.5, 2.5, 3.5 and 4.5 (%w/v) aqueous dispersions of *M. flagellipes* seed gum. The test was repeated three times in each case and the average taken. Absolute viscosities of the *M. flagellipes seed* gum dispersions were calculated using equation 2.

$$\frac{\eta_2}{\eta_1} = \frac{\rho_2 \, t_2}{\rho_1 \, t_1} \quad \ldots\ldots\ldots \quad (2)$$

Where is η is absolute viscosity, ρ is density and t is time. The subscripts 1 and 2 represent *Mucuna*

flagillepes seed gum aqueous dispersion and deionized water, respectively.

Determination of specific gravity

A 3 g quantity of the *M. flagellipes* seed gum was weighed (W_1). Then, 10 ml measuring cylinder was also weighed (W_2), and 5ml of distilled water introduced into it and re-weighed (W_3). Then, the 3 g gum was wrapped in small transparent polyethylene water-proof leather and introduced into the cylinder and re-weighed (W_4). The volume of water displaced was noted and the weight was calculated. The weight of the volume of water displaced by the gum is the weight of equal volume of water. Thus, specific gravity was calculated from equation 3.

$$Specific\ gravity = \frac{Weight\ of\ substance}{Weight\ of\ equal\ volume\ of\ water} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Temperature change on storage

The temperature values of the different concentration of the aqueous dispersions of the *M. flagellipes* seed gum were determined for 3 weeks. The temperature was recorded after 1 week, 2 weeks and 3 weeks to check stability of aqueous dispersions of the gum.

Solubility Test

A 0.2 g quantity of pulverized *M. flagellipes* seed gum was introduced into ten test tubes. Then six polar solvents (distilled water, acetone, methanol, acetic acid, ethyl acetate and formic acid) and four non-polar solvents (diethyl ether, carbon tetrachloride, benzene and toluene) were each used to make the primary solution and each was made up to 10 ml. They were left for 24 h at room temperature and then the solubility of the gum was determined.

Characterization of the PEGylated *Mucuna flagellipes* seed gum

Determination of pH

The aqueous dispersions of the 1:1, 1:2, 2:1, 1:3, 3:1 and 2:3 of gum-PEG ratios (PEGylated gum) were prepared and the pH determined using the pocket sized digital pH meter (HANNA Instruments, India).

For true density, absolute viscosity, specific gravity, temperature on storage and solubility test, the methods described for the characterization of the unPEGylated *Mucuna flagellipes* seed gum above were also adopted. They were carried out using 0.5, 1.5, 2.5, 3.5 and 4.5 (% w/v) of the 4:2 ratio PEGylated gum.

Experimentally induced excision wounds

The animal experimental protocols were in accordance with the guidelines for conducting animal experiments stipulated by our Institution's Animal Ethics Committee and in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC) [47]. Sixty Sprague-Dawley albino rats (240 – 280 g) of both sexes were obtained from of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, housed and provided with standard feed and water *ad libitum*. The rats were divided into ten groups of six rats each. They were fed for one week and allowed to acclimatize to laboratory condition. The animals were anesthetized with ketamine® i.m injection (30 mg/kg, 100 mg/ml) prior to creation of the wounds. The wound site was prepared following the excision wound model [48]. The hair around the dorsum of the rats were shaved with sharp surgical scissors and swabbed with cotton wool. A paper disc of 2 cm was placed on the shaved dorsum of the rats and traced with the ink marker. Full thickness wounds were inflicted by excision following the ink mark on the skin of the shaved dorsum part of the rats, with sterile surgical scissors and surgical blades. Then groups II-IX were swabbed with cotton wool soaked in methylated spirit, and immediately treated with the unPEGylated gum (1:0), different ratios of the PEGylated gum (1:1,1:2, 2:1, 1:3, 3:1, 2:3) and PEG alone (0:1), respectively. Group I (gum:PEG, 0:0) was not treated with any agents but swabbed with methylated spirit to avoid wound infection, thus used as negative control. Group X (cicatrin) was treated with Cicatrin powder as positive control after swabbing with methylated spirit.

Treatment and measurement of wound diameter

Treatment and measurement were repeated every three days. For each group, the wounds were cleaned with cotton soaked in methylated spirit (to avoid wound infection) and the wound diameter measured before applying the agents to the wounds and thereafter every three days. The wound diameter measurements were taken in triplicate and the average taken. After treatment, the rats per group were separated for some time

to prevent the agents from sticking to the wounds and to prevent the rats from leaking one another's wound and the agent applied. Treatment and measurement were continued till 18 days.

Determination of wound closure rate or Percentage wound healing

The wound closure rate (or percentage wound healing) was calculated for each batch using equation 4.

$$Wound\ closure\ rate\ OR$$
$$Wound\ healing\ (\%) = \frac{L_o - L_f}{L_o}\ X\ 100 \quad \ldots\ldots (4)$$

Where L_o is the lengths of the originally created wound; L_f the length of the wound for a specified time interval.

Toxicity studies of the wound

This study was carried out on each animal by assessing the following parameters on the rats: dryness of wound area, wound odor, exudation, wound contraction, effect of food intake, effect of water intake, itching and physical state.

Statistical analysis

All values were reported as mean±S.E.M. Statistical significance of differences among groups were assessed using one-way ANOVA. Value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

White hydrophilic *Mucuna flagellipes* seed gum was obtained on precipitation with acetone. The white gum turned faint white colour after drying, milling and sieving into fine powder. The extraction process afforded 34.35 % yield of the aqueous extract, which is very encouraging. White to off-white fine powder (whiter than unPEGylated) was obtained after incubation, evaporation, drying, milling and sieving. The results of some physicochemical properties of unPEGylated and PEGylated *Mucuna flagellipes* seed gum are shown in Figures 2 and 3, respectively. The pH of unPEGylated gum was in the range of 4.3 - 4.7 while that of PEGylated gum ranged from 5.5 to 5.7, indicating that the pH of PEGylated gum is much more weakly acidic than the pH of the unPEGylated gum dispersion in water. By implication, they could be useful excipients in the formulation of weakly acidic drugs. The true density of unPEGylated gum ranges from 0.224 to 2.016 g/cm^3 whereas the true density of PEGylated gum ranges from 0.89 to 0.99 g/cm^3. It was obvious from this result that the density of the gum increased with increase in concentration of the gum. This is important and has to be considered when the gum dispersions are to be used as pharmaceutical suspension stability enhancer, as reported elsewhere [30]. Also, although the density of both PEGylated and unPEGylated *Mucuna flagellipes* seed gum increased with increase in concentration of the gum in the dispersion, they differed in values showing that PEG has exerted effects on the pH property of the gum. Both are pharmaceutically important, and should be considered where the gum dispersions are to be used such as suspension formulation and formulation of syrup that needs different liquid ingredients. Similarly, the specific gravity of the aqueous dispersions of the 4:2 gum-PEG ratio PEGylated gum also increased with increase in concentration, but differed in values from unPEGylated gum. It ranged from 0.116 for 0.5 %w/v to 1.047 for 4.5 %w/v (PEGylated gum) and from 0.25 for 0.5 %w/v to 1.875 for the 4.5 %w/v aqueous dispersion of unPEGylated gum. This means that PEGylation actually affected the specific gravity values of the gum. This may be of pharmaceutical importance in checking how the dispersion concentrates or dilutes relative to water or during formulation of pharmaceutical semi-solid formulations such suspensions [30, 33]. The absolute viscosity of unPEGylated gum ranges from 0.524 to 15.23 Poise whereas the absolute viscosity of PEGylated gum was in the range of 2.268 -5.405 Poise. From the result, it was clear that absolute viscosity increased as the concentration of the gum in the dispersions increased. Thus, it represents the capability of the *Mucuna flagellipes* seed gum in solution to enhance the viscosity of the solution. Comparatively, the unPEGylated gum had higher absolute viscosity value than the PEGylated gum especially at high concentration of gum in the dispersion. The difference in the values could be attributed to the degree of gum-PEG interaction. It showed that PEGylation altered the viscosity and thus enhanced the pouring ability especially at high concentration. This is of tremendous significance in uniform dosage withdrawal from semi-solid dosage forms containing the PEGylated gum dispersion, similar to reports on polyherbal formulations [27 – 29]. Figures 4 and 5 show the time-resolved temperature-dependent stability of unPEGylated *Mucuna flagellipes* seed gum aqueous dispersions and PEGylated gum, respectively.

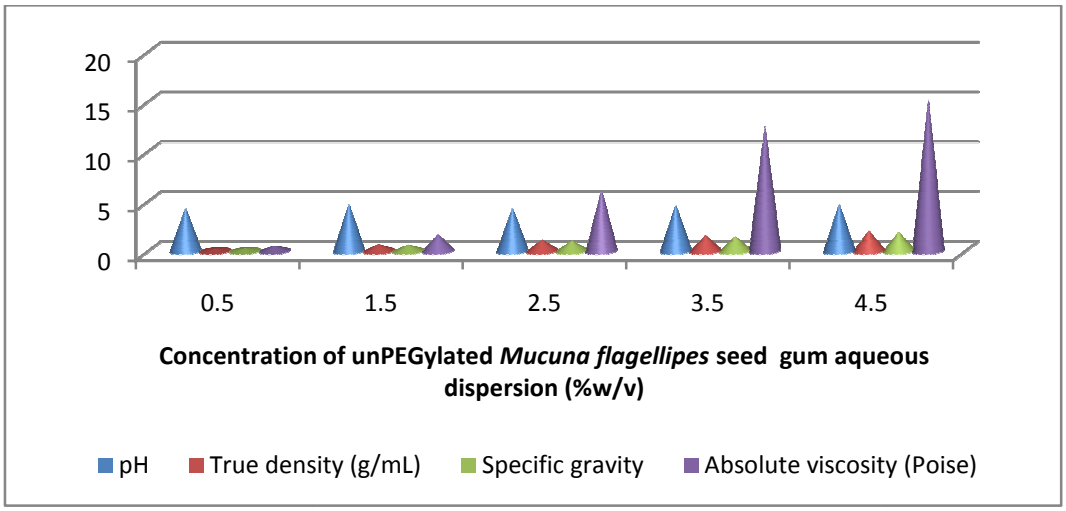

Figure 2: Physicochemical properties of unPEGylated Mucuna flagellipes seed gum

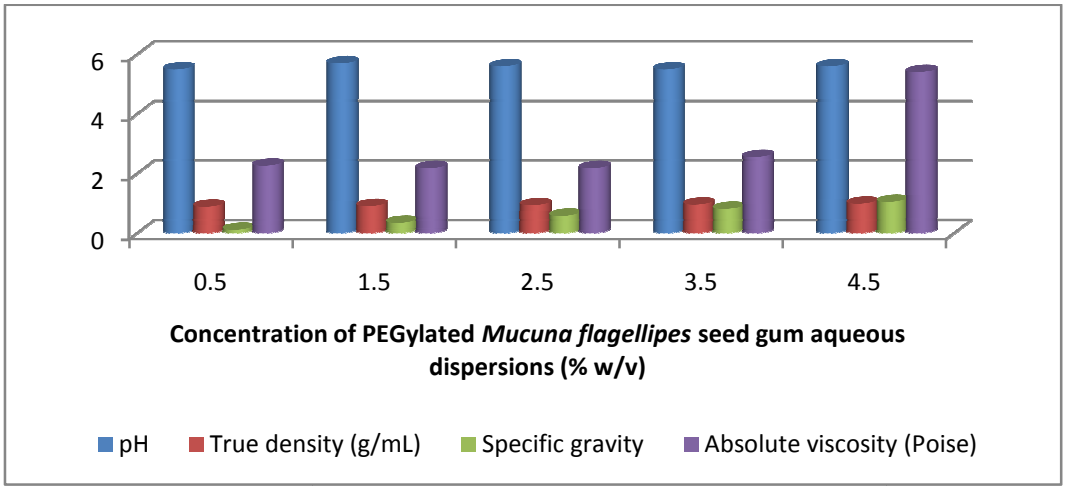

Figure 3: Physicochemical properties of PEGylated Mucuna flagellipes seed gum

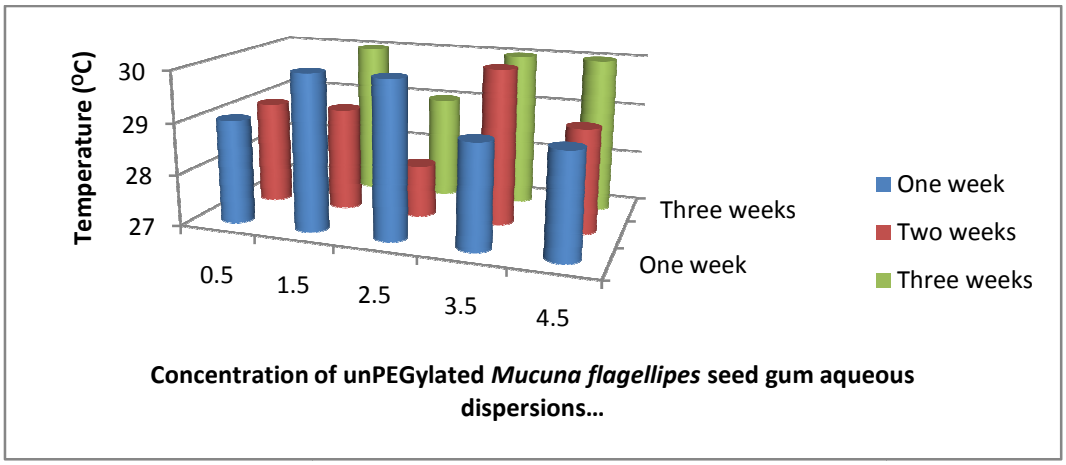

Figure 4: Time-resolved temperature-dependent stability of unPEGylated Mucuna flagellipes seed gum aqueous dispersions

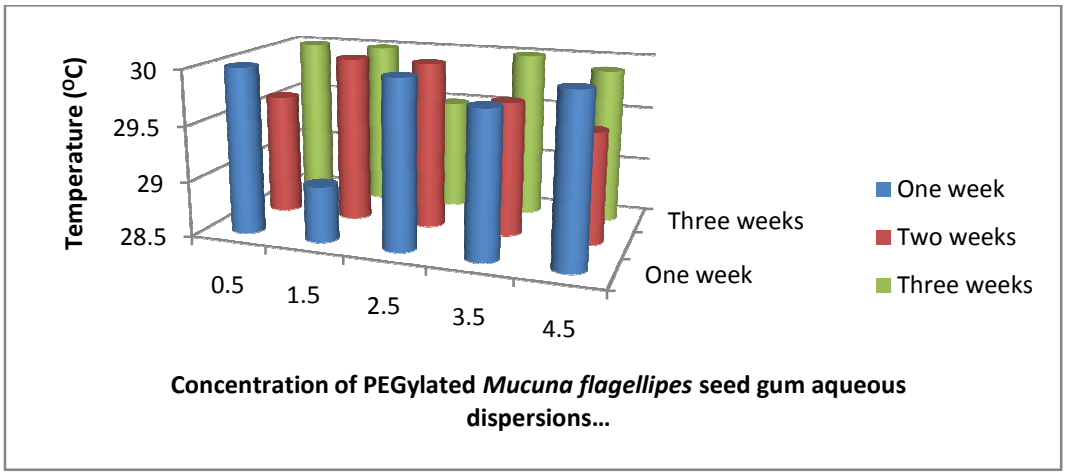

Figure 5: Time-resolved temperature-dependent stability of PEGylated Mucuna flagellipes seed gum aqueous dispersions

Table 1: Solubility of PEGylated and unPEGylated *Mucuna flagellipes* seed gum

Solvent	UnPEGylated gum	PEGylated gum
Distilled water	+++++	+++++
Acetone	-	-
Methanol	+	+
Formic acid	+++++	+++++
Ethyl acetate	-	-
Acetic acid	+	+
Diethyl ether	-	+
Carbon tetrachloride	-	-
Benzene	-	++
Toluene	-	-

Key: +++++ = very soluble; - = insoluble; + = slightly soluble

Table 2: Effects of treatment (wound closure in cm) with exposure time

Gum-PEG Ratio	Day One	Day Three	Day Six	Day Nine	Day Twelve	Day Fifteen	Day Eighteen
00:00	2.0 ± 0.0	1.93 ± 0.017^{b}	1.83 ± 0.017^{b}	1.62 ± 0.017^{b}	0.9 ± 0.058^{b}	0.43 ± 0.033	0.23 ± 0.00
01:01	2.0 ± 0.0	1.88 ± 0.017^{b}	1.82 ± 0.017^{b}	1.67 ± 0.033^{ab}	0.8 ± 0.058^{b}	0.5 ± 0.058	0.15 ± 0.029
01:01.5	2.0 ± 0.0	1.8 ± 0.058^{ab}	1.7 ± 0.00^{ab}	0.87 ± 0.033^{a}	0.3 ± 0.029^{ab}	0.0 ± 0.00^{a}	0.0 ± 0.00
01:02	2.0 ± 0.0	1.9 ± 0.029^{b}	1.8 ± 0.00^{ab}	1.00 ± 0.00^{a}	0.63 ±0.033^{ab}	0.28 ± 0.017^{ab}	0.0 ± 0.00
02:01	2.0 ± 0.0	1.88 ±0.017^{ab}	1.78 ±0.017^{ab}	0.9 ± 0.058^{a}	0.52 ± 0.044^{a}	0.23 ± 0.033^{ab}	0.0 ± 0.00
01:03	2.0 ± 0.0	1.9 ± 0.0^{b}	1.73 ±0.033^{ab}	1.2 ± 0.058^{ab}	0.8 ± 0.029^{b}	0.4 ± 0.00	0.17 ± 0.017
03:01	2.0 ± 0.0	1.7 ± 0.0^{a}	1.6 ± 0.00^{a}	1.1 ± 0.058^{ab}	0.65 ±0.029^{ba}	0.3 ± 0.058^{ab}	0.0 ± 0.00
01:00	2.0 ± 0.0	1.7 ± 0.0^{ab}	1.65 ± 0.00^{ab}	0.95 ± 0.029^{a}	0.45 ± 0.029^{a}	0.33 ± 0.017^{ab}	0.1 ± 0.00
00:01	2.0 ± 0.0	1.9 ± 0.0^{b}	1.77 ±0.033^{ab}	1.25 ± 0.029^{a}	0.87 ± 0.033^{b}	0.45 ± 0.029^{b}	0.22 ± 0.011
Cicatrin	2.0 ± 0.0	1.7 ± 0.058^{ab}	1.58 ± 0.044^{a}	0.9 ± 0.00^{ab}	0.5 ± 0.00^{a}	0.25 ± 0.029^{a}	0.0 ± 0.00

Key: 'a' showed significance ($p < 0.05$) when compared with the negative control, 'b' showed significance ($p < 0.05$) when compared with positive control

Table 3: Percentage wound closure on the rat for the period of study

Groups	Gum-PEG ratio	DAY 1	DAY 3	DAY 6	DAY 9	DAY 12	DAY 15	DAY 18
I	0:0	0.00	3.50	8.50	19.00	55.00	78.50	88.50
II	1:1	0.00	6.00	9.00	16.50	60.00	75.00	92.50
III	1:1.5	0.00	10.00	15.00	56.50	85.00	I00.00	I00.00
IV	1:2	0.00	5.00	10.00	50.00	68.50	86.00	100.00
V	2:1	0.00	6.00	11.00	55.00	74.00	88.50	100.00
VI	1:3	0.00	5.00	13.50	40.00	60.00	80.00	91.50
VII	3:1	0.00	15.00	20.00	45.00	67.50	85.00	100.00
VIII	1:0	0.00	13.50	17.50	52.50	77.50	83.50	95.50
IX	0:1	0.00	5.00	11.50	25.00	56.50	77.50	89.00
X	cicatrin	0.00	15.00	21.00	55.00	75.00	87.50	100.00

Table 4: Toxicity profiles of the samples based on the excision wound study.

Group	Ratio of gum to PEG	Dryness of wound area	Wound odour	Exudation	Wound contraction	Effect of food & water intake	Itching	Physical state
I	0:0	+	+++	++	+	+	+++	+
II	1:1	++	-	+	++	+	++	++
III	1:1.5	+++	-	-	+++	++	+	+++
IV	1:2	+++	-	-	+++	++	++	+++
V	2:1	+++	-	-	+++	++	++	+++
VI	1:3	++	-	-	++	+	+	++
VII	3:1	+++	-	-	+++	++	++	+++
VIII	1:0	+	+	-	++	+	+	++
IX	0:1	+	-	-	++	+	++	++
X	cicatrin	+++	-	-	+++	++	++	+++

Key: +++ = high intensity; ++ = medium intensity; + = low intensity; - = no effect

For the three weeks of temperature-dependent study, the temperature values of unPEGylated gum aqueous dispersions were found to be within ambient temperature range and the variations were negligible. This implies that the gum dispersions are relatively stable and maintains room temperature upon storage, provided it is tightly closed in amber-coloured plastic or glass container. In comparison with the unPEGylated gum, the PEGylated gum showed more temperature stability. It was within ambient temperature range and did not show variations like the unPEGylated gum. Thus, the PEGylated gum forms a better choice for use in pharmacy than the unPEGylated gum. Moreover, the solubility profiles of unPEGylated and PEGylated *Mucuna flagellipes* seed gum at room temperature are presented in Table 1. The result indicates that *Mucuna flagellipes* seed gum and PEGylated gum are more soluble in polar than non-polar solvents, but not soluble in non-aqueous or organic solvents. They are highly soluble in water and formic acid, but slightly soluble in methanol and acetic acid. This result, which is consistent with the physiochemical properties of *Mucuna flagellipes* as reported by Odedele [30], is of importance in the choice of dispersing vehicle during production of the formulations involving the gum dispersion. It was further realized from the solubility study that PEGylated gum dissolved in both polar and non-polar solvents. Just like the unPEGylated gum, the PEGylated gum was highly soluble in water and formic acid and slightly soluble in methanol, acetic acid but unlike unPEGylated gum, it was sparingly soluble in benzene and

diethyl ether. It is insoluble in some organic solvents such as carbon tetrachloride and toluene. The reason for the solubility of PEGylated gum in the organic solvent is unknown but may be related to PEGylation.

The wound healing effect of unPEGylated and PEGylated *Mucuna flagellipes* seed gum, PEG 6000 as well as cicatrin® powder are depicted in Table 2 (wound closure with exposure time) and Table 3 (percentage wound closure on the rat with exposure time). Results indicate that the wound healing activity of the formulations was much more than that of the negative control (untreated). In majority of cases, there was general decrease in wound area with exposure time after the application of the formulations. While the wound closure times of PEGylated *Mucuna flagellipes* seed gum were less the percentage wound closure upon application of the PEGylated *Mucuna flagellipes* seed gum were more than those of cicatrin powder (positive control). In all the formulations, the individual component (unPEGylated *Mucuna flagellipes* seed gum or PEG 6000) when used alone showed a better activity than the negative control (untreated) but the combination (PEGylated *Mucuna flagellipes* seed gum) showed a better wound healing activity than the individual components. At day 18 post-wounding, a significant reduction ($p < 0.001$) in wound contraction was observed among all treated groups with a maximum reduction ($p < 0.001$) in group III (PEGylated gum containing 01:01.5 ratios of mucuna gum and PEG 6000). Furthermore, continuous application of the formulations at wound sites caused progressive acceleration in mound closure at day 18 post-wounding (Table 2). By implication, continuous application of the formulations has positive effect on wound closure. The result indicates that there was gradual decrease in wound size as the time prolongs. Overall, group III containing PEGylated mucuna gum (01:01.5 ratios of mucuna gum and PEG 6000) had the greatest decrease in wound while the least healed was group I which had no treatment.

From the result of the wound healing study, it was observed that all the groups formed scab after three days except group I (0:0), group IV (1:2), group VI (1:3) and group IX (0:1). Rats in some groups had the scab fall off by day 6,while some others fell off on day 9 all exposing the wound. After the fall off of the scab and administration of the different gum-PEG ratio, epithelialization sets in. The gum absorbs the exudate, helps to debride the wound and enhances healing, which is in consonance with earlier report on wound healing [7-11]. In comparison between groups, group III (1:1.5) showed a significant difference ($p<0.05$) on days 3, 6, 9 and 12, but a marked significant difference on the 15th day compare to the positive control (group X or cicatrin®), negative control (group I or 0:0) and other groups. Group VIII (1:0 or gum alone) showed significant difference ($p < 0.05$) on days 3, 6, 9, 12 and 15 compared to the negative control, but insignificant ($p > 0.05$) on days 6 and 12 compared to the positive control. It is also insignificant compared to group III. The analysis confirmed that group III (1:1.5 gum-PEG ratio) was more potent than the positive control and was due to PEGylation at optimal Gum-PEG ratio and interaction. Groups IV and VII also showed significant difference ($p < 0.05$) compared to positive and negative controls, but not as optimal and potent as group III (1:1.5 Gum-PEG ratio).

Reports have shown that wound healing is mediated through various phases by a wide range of chemically co-ordinate cellular processes as well as hormonal influences [17, 18]. Many plants and plant constituents have shown good wound healing activity, which were attributed to flavonoids contained in the plant [23 – 24, 26]. Previous studies on the phytochemical constituents on *Mucuna flagellipes* seed gum showed that it contains flavonoids and other anti-oxidant phytoconstituents [30, 34]. The anti-oxidants present in *Mucuna flagellipes* could be responsible for the observed wound healing effect, especially on groups treated with *Mucuna flagellipes* seed gum-containing products (groups II - VIII). This is because, the mechanisms of wound healing might be attributed to stimulation of the production of antioxidants in wound site and which provides a favorable environment for tissue healing and these antioxidants may play a significant role in the wound healing process and may be important contributory factor in the wound healing property [10]; antioxidants also improve wound healing and protect tissues from oxidative damage [4, 6, 8]. In addition, flavonoids can scavenge for the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol and these reactive intermediates are potentially implicated in delayed wound healing [9], thus the higher the flavonoids content, the stronger the antioxidant activity [16, 49].

Various grades of polyethylene glycol (PEG) have been employed in drug delivery; PEG 6000 has

been added to honey and pollen extract for the treatment of test lesions in the cow [50]; PEG 4000 has been employed to potentiate the wound healing effect of *Vernonia amygdalina* [44]. It has been proposed that the mechanism of wound healing by polyethylene glycols (PEGs) is by promoting non-adherent healing through polymer coating or siliconization of the injured surface [51]. Recent report indicates that PEG 6000 has been extensively used for mucoadhesive applications due to its ability to exhibit high adhesive bond strengths in contact with tissues [52], which increases residence time and drug bioavailability. Thus, combination of PEG 6000 with other mucoadhesive agents such as gums would improve the functionality of the agents in pharmaceutical formulations, causing an increase in mucoadhesion and making the incorporated drug available at the delivery site [44 – 45, 50 – 52]. In this study, PEGylated *Mucuna flagellipes* seed gum exhibited synergistic effect due to the combined wound healing effect of PEG 6000 and *Mucuna flagellipes* seed gum coupled with the additive mucoadhesive effect at the site of action. In all ratios, there was a progressive increase in rate of wound healing and this was due to the contributory action of the combined gum and PEG to wound healing. However, the 1:1.5 ratio PEGylated gum healed the wound faster than cicatrin powder; it healed the wound after 15 days, while cicatrin healed after 18 days. Thus, PEGylated *Mucuna* gum could be used as wound healing agent especially at optimal gum-PEG ratio (1:1.5). This study has shown that PEGylated mucuna gum has values in management of superficial skin wounds.

The toxicity evaluation of the wound was to determine which of the treatments causes less discomfort and challenge to the rats. Results depicted in Table 4 indicate that the formulations are safe.

CONCLUSIONS

In this study, the drug delivery and therapeutic (wound healing) potentials of unPEGylated and PEGylated *Mucuna flagellipes* seed gum was evaluated using the excision wound model in rats. Results indicate that PEGylated gum significantly accelerated wound healing compared with antibiotic powder Cicatrin (positive control), unPEGylated gum and PEG 6000 alone. Overall, PEGylated *Mucuna* gum could be used as wound healing agent especially at optimal gum-PEG ratio (1:1.5). We therefore recommend this formulation as an agent for wound management.

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