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Nicotiana tabacum (Tobacco) Promotes Wound Healing in Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author RSA conceptualized and designed the study, supervised the experiment, analyzed and interpreted the results and prepared the manuscript. Author OTB performed the experiment, collected the data and performed initial analysis of the data. Both authors RSA and OTB did the literature search, read and approved the manuscript. Author RSA is the guarantor of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Objective: The health hazards of tobacco smoking and diabetes mellitus constitute major and diverse global burden. The influence of *Nicotiana tabacum* (tobacco) powder and ethanolic extract on excised wound of diabetic rats was investigated.

Methods: The wounds of diabetic adult male wistar rats in groups of six were dressed with tobacco powder (TPD), tobacco ethanolic extract (TED), sofratulle (SD) and normal saline (ND) daily till healed. There were composite control groups namely TPC, TEC, SC and NC.

Every three days, the wound areas were measured in order to calculate the mean wound contraction rates. Granulation tissue was biopsied from an animal per group on day 3,6 and 9 for histopathological evaluation and after healing, the scars of the remaining animals were biopsied for histology.

Results: On day 3, the tobacco powder diabetic (TPD) group had the highest mean wound contraction rate and even higher than its control group. At day 18, the tobacco extract control group had the least mean wound contraction rate. The mean wound contraction rates of some diabetic groups were significantly higher than those of the respective control on day 3 and 12 (TPD vs TPC; TED vs TEC). The TPD mean wound contraction rates were significantly higher than

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those of ND on day 3,6,9 and 12. Histology of the granulation tissues of the tobacco diabetic groups was similar to those of the other groups. Sections of the wound scars revealed stratified squamous epithelia with abundant collagen fibres and blood vessels in all the groups. It was also observed that the scars were more fibrous than cellular with dermal appendages observed in some of the groups.

Conclusion: Topical application of *Nicotiana tabacum* (Tobacco) promotes wound healing with scars comparable to those of rats dressed with sofratulle.

Keywords: Nicotiana tabacum; powder; ethanolic extract; diabetes mellitus.

1. INTRODUCTION

Traditionally, since ancient times, herbal formulations (preparations) have been used to treat human ailments with varying outcome. A considerable number of drugs are being developed from various parts of plants of diverse types and species [1].

Nicotiana tabacum is a herbaceous plant widely cultivated in many countries including Nigeria. The main derivative of its leaves known as tobacco is consumed in form of snuff or cigarette for social and recreational purposes. According to the World Health Organization (WHO) estimates, over 1.1 billion people world-wide consume tobacco. Also tobacco has been documented to be the greatest risk for preventable mortalities. Nicotine, an alkaloid has been identified to be responsible for the morbidities and mortalities associated with tobacco consumption [1] and it constitutes 4-8% of the dried leaves [2].

Diabetes mellitus is a systemic metabolic disease that derails the functions and integrity of vital structures and organs such as the brain, kidney, eyes, heart, arteries and liver. Foot ulcers secondary to diabetes heal poorly and invariably become chronic. Many surgical dressing materials are available to effectively manage diabetic foot ulcers. However, due to relatively prohibitive cost arising from long duration of management coupled with low socioeconomic status of most of our patients, these dressing materials are more often than not out of reach of most of the patients. Thus the need to find cheaper, affordable but effective alternatives becomes pertinent. Exploring the wound healing potentials of *N. tabacum* will be an affordable and safe alternative in the utilization of tobacco. This plausible alternative pathway for tobacco utilization if commercialized, will reduce the incidence of tobacco related morbidity and mortality. Bearing this in mind, the current study was consequently designed and performed.

2. MATERIALS AND METHODS

2.1 Plant Materials

2.1.1 Collection and identification of plant materials

Fresh leaves of *Nicotiana tabacum* were sourced from a commercial tobacco plantation situated in Iseyin, Oyo north, Nigeria. Specie identification and authentication were done at the Herbarium of Botany Department, University of Ibadan, Nigeria and a specimen deposit with voucher no UIH-226021 was made.

2.1.2 Preparation of powder and extract

The leaves were initially washed under running water and subsequently left to dry under ambient laboratory temperature. Thereafter, they were blended into smooth, fine textured and non-granular powder using an electric grinder and a portion used for ethanolic extraction while the remaining preserved for topical application.

2.1.2.1 Phytochemical analysis

By means of previously described techniques [3-6], the phytochemical analyses of *N. tabacum* for alkaloids, saponins, tannins, flavonoids, anthraquinones, steroids and cardiac glycosides, were done with 8 g of the powder.

2.1.2.2 Ethanolic extraction

Two hundred and fifty grams (250 g) of the powder was used for the ethanolic extraction with 100% ethanol. A yield of 12.35% was obtained and stored under optimal conditions till application.

2.2 Animals

Forty eight healthy inbred adult male wistar rats weighing between140 and 200 g sourced from the College of Medicine, University of Ibadan central animal house were used for the study.

They were initially acclimatized in a well ventilated and illuminated environment with conducive ambient temperature of 20 to 26°C for two weeks. They had liberal standard rat diet and water for the entire period of the study. The animals were closely monitored and those that developed features of sepsis were withdrawn from the study. Animal handling was in compliance with the guidelines prescribed by the ethical conduct of animal research of the University of Ibadan. Also, the principles of laboratory animal care as contained in the 8th edition (2011) of the Guide for the Care and Use of Laboratory Animals by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals were observed [7].

2.3 Design of the Experiment

The parameters for group allotment were induced diabetes mellitus and dressing material. Consequently, the animals were randomly allotted to eight groups of six each. Only four of the groups had induced diabetes and the four non-diabetic groups served as respective control.

1. Tobacco powder control (TPC)
2. Tobacco powder diabetic (TPD)
3. Tobacco extract control (TEC)
4. Tobacco extract diabetic (TED)
5. Sofratulle control (SC)
6. Sofratulle diabetic (SD)
7. Normal saline control (NC)
8. Normal saline diabetic (ND)

2.4 Induction of Diabetes Mellitus

The pre induction fasting blood sugar levels were estimated with single touch glucometer (ACCUCHECK®, Roche Diagnostics, Germany) using the blood obtained from the tails of the rats. The baseline pre induction blood sugar level ranged from 55- 75/dl. Based on the outcome of previous studies, a single dose of 100 mg/kg body weight of alloxan monohydrate dissolved in normal saline and administered intraperitoneally was used to induce diabetes mellitus. A 72 hour post induction fasting blood sugar level above 250 mg/dl was considered diabetic [8].

2.5 Wound Creation

After prior sedation with intramuscular ketamine hydrochloride (120 mg/kg); the dorsolateral skin of each rat was cleansed with savlon antiseptic liquid and a 2 cm by 2 cm full thickness skin about 1.5 cm from the vertebral column was excised thus establishing an excisional wound.

2.6 Wound Management and Data Collection

The tobacco powder, tobacco extract, sofratulle and normal saline was used as wound dressing material for the respective paired group- TPC, TPD; TEC, TED; SC, SD; and NC, ND.

Wound dressing was done daily. However, before change of dressing, wound size estimation was done by taking dimensions along two perpendicular plane. The values obtained were used to derive the contraction rates in percentages and this was repeated every 3 days.

Granulation tissue biopsy was taken from a member of each group on day 3,6 and 9. These samples were processed for histological evaluation using Haematoxylin and Eosin stain.

These slides were used for evaluation of wound healing in terms of cellularity, angiogenesis, fibroplasia and collagen synthesis. The excision of granulation tissue served as the exit point for such animals and the wounds of remaining animals in all the groups were allowed to heal. The resultant scars were similarly processed for light microscopy. Falling off of the eschar without any residual wound indicated the endpoint of complete epithelization and the days required for this connoted the duration of healing.

2.7 Data Analysis and Processing

The numerical aspects of the results were analyzed with Statistical Package for the Social Sciences (SPSS) version 24 and expressed as percentages, means plus standard deviation of means (SD). The student t-test was used for inter group comparison and level of significance was set at $p<0.05$.

3. RESULTS

Nicotiana tabacum leaves in both powder and ethanolic extract formulations tested positive for terpenoids, saponins, tannins, alkaloids, steroids anthraquinones and flavonoids. Cardiac glycosides were not detected in either of them (Table 1). Quantitative wise, the ethanolic extract had 4.95% flavonoids, saponins 7.4% and alkaloids 4.20%. All the diabetic sub groups were hyperglycemic as evidenced by the range of the

mean fasting blood sugar levels (354.2- 390 mg/dl). The range was maintained at repeat fasting blood sugar level estimations of day 12 and 18 of the study. A downward trend was observed in the mean body weights of all the diabetic groups with the pre induction mean body weight being the highest and the day 18 (post induction) being the least. The mean body weight was largely on the increase amongst the control groups (Table 2). On day 3, the tobacco powder diabetic (TPD) group had the highest mean wound contraction rate and even higher than its control group (TPC) however by day 6, the sofratulle control group (SC) had the highest rate and all the diabetic groups had lower rates than the respective control (Table 3). The day 9 results revealed that three groups i.e powder control (TPC), sofratulle groups (SC and SD) had mean wound contraction rates greater than 60%. As of day 12, only the tobacco extract control (TEC) and normal saline diabetic (ND) groups had a mean contraction less than 50% while the TPC, SC and SD groups had rates above 80%. By day 15, healing had virtually occurred in groups TPC, SC, SD and NC as evidenced by mean wound contraction rates greater than 90% and substantially in groups TPD and TED while the rate was barely 50% in TEC. The tobacco extract control group had the least mean wound contraction rate (63.25±5.77%) (Table 3).

3.1 Mean Wound Contraction Rates Comparisons

We observed that the mean wound contraction rates of some diabetic groups were significantly higher than those of the respective control on day 3 and 12 (TPD vs TPC; TED vs TEC). While the controls were significantly higher than the respective diabetic on day 3,6,9 &12. (TED vs TEC; ND vs NC; TPD vs TPC). The difference between tobacco powder diabetic and sofratulle diabetic was only significant on day 12. We however, noted that the TPD mean wound contraction rates were significantly higher than those of ND on day 3,6,9 & 12. Comparison between the diabetic groups of the tobacco powder and extract i.e. TPD vs TED showed that the former was significantly higher than the latter on day 3; while the converse but insignificant were the case on day 6,9 and 12. The differences in the mean rates between groups TED and SD were significant on day 3 and 12. However, for groups TED and ND, significant differences were observed on day 6,9 and 12. Not beyond expectation, the mean rates of the tobacco powder control were significantly higher than those of ND on day 3,6,9 and 12. Very marginal differences were observed between TPC and SD throughout the duration of the study. The mean rates for the TED group were significantly lower than those of the SC group on day 3,6,9 and 12; whereas for TED vs NC significant mean rate differences were at day 3 and 12. The mean wound contraction rates for the TEC were significantly lower than those of the SC group on day 3,6,9 &12 while it was only of day 12 for group NC. It should be noted that the SD rates were significantly higher than those of the ND group on day 6,9 and 12. Finally, only on day 3 and 12 were the SC rates significantly higher than those of the NC (Table 4).

3.2 Histology of Granulation and Scar Tissues

Inflammatory cells were observed in all the granulation tissue biopsies from all the groups on day 3; however, they were more intense in groups TPC and TEC than the respective diabetic groups. New blood vessels, fibroblasts, macrophages and fibrillary structures were also observed (Plate 1). By day 6, multilayered squamous cells suggestive of stratified epithelial linings were observed at the periphery of the biopsied granulation tissues of groups TPD and TED. Other day 6 findings were collagen, fibroblasts and blood vessels (Plate 2). Increased fibroblasts and collagen deposits were observed in the granulation tissue of all the groups at day 9 besides the considerable cellular infiltration of diverse types (Plate 3). Sections of the wound scars revealed stratified squamous epithelia with abundant collagen fibres and blood vessels in all the groups. It was also observed that the scars were more fibrous than cellular. Dermal appendages were observed in some of the groups (Plate 4).

Table 1. Phytochemical analyses N. *tabacum* powder and extract

Anthraquinones	Terpenoids	Alkaloids	Flavonoids	Tannins	Steroids	Saponins	Cardiac glycosides
Powder + Extract +	Powder + Extract +	Powder + Extract +	Powder + Extract +	Powder + Extract +	Powder + Extract +	Powder + Extract +	Powder - Extract -

+ (present) and – (absent)

Table 2. Mean fasting blood sugar levels and mean body weight

Parameter	TPC	TPD	TEC	TED	SC	SD	NC	ND
Pre induction sugar level (mg/dl)	N/A	62.67	N/A	65.50	N/A	59.83	N/A	62.67
Post induction sugar level (mg/dl)		390		354.2		353.8		370.8
Pre induction weight (g)	167.00±7.90	199.17±21.10	162.00±11.71	185.67±14.29	160.00±21.12	189.00±22.86	158.67±14.38	191.67±10.35
Post induction weight-day 6	171.00±8.51	186.75±30.58	170.25±25.42	171.00±9.00	159.20±23.39	168.33±9.07	169.60±15.40	183.33±5.77
Post induction weight-day 12	170.25±6.95	186.33±29.69	172.00±20.05	171.33±12.66	167.67±20.84	166.33±8.08	169.75±16.15	182.00±16.97
Post induction weight-day 18	172.33±11.93	182.33±27.74	173.25±21.03	169.00±5.66	165.33±22.05	161.33±7.23	173.00±3.46	177.50±10.61

NA= Not applicable. The control groups were not induced; the weights were itemized just to show the trend. The blood sugar levels in all the diabetic groups were markedly elevated and significantly higher (P≥ 0.05) than the pre induction levels

Table 3. Interval mean values of wound contraction rates in percentages (%)

Group	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
TPC	12.31±3.28	32.89±12.70	63.22±14.45	80.85±4.92	93.74±2.97	96.74±2.86
TPD	16.55 ±3.44	25.24±4.62	43.48±10.80	58.54±32.61	76.38±13.07	93.55±6.21
TEC	9.00±4.01	21.40±14.97	34.68±11.93	44.64±3.50	49.29±6.29	63.25±5.77
TED	4.97±1.85	26.21±4.26	46.27±5.01	61.13±2.92	85.40±3.15	92.95±1.23
SC	14.31±3.38	36.07±9.56	66.68±5.01	85.40±3.24	97.01±1.03	99.01±1.34
SD	11.34±6.76	30.72±10.37	60.67±16.00	80.87±13.83	94.64±3.85	97.9±2.04
NC	9.59±3.62	27.89±9.67	51.83±18.42	75.17±5.70	94.14±1.48	98.99±1.75
ND	6.35±3.01	15.19±7.17	22.97±8.91	37.87±8.23	56.04±6.50	70.63±10.27

Table 4. Intra and Inter group comparisons of wound mean contraction rates

Compared groups	Day 3 mean rates	Day 6 mean rates	Day 9 mean rates	Day12 mean rates
TPD vs SD	16.55±3.44	25.24±4.62	43.48±10.80	58.54±32.61*
	11.34±6.76	30.72±10.37	60.67±16.00	80.87±13.83
TPD vs ND	16.55±3.44*	25.24±4.62*	43.48±10.80*	58.54±32.61*
	6.35±3.01	15.19±7.17	22.97±8.91	37.87±8.23
TPD vs TED	16.55±3.44*	25.24±4.62	43.48±10.80	58.54±32.61
	4.97±1.85	26.21±4.26	46.27±5.01	61.13±2.92
TED vs SD	4.97±1.85*	26.21±4.26	46.27±5.01	61.13±2.92*
	11.34±6.76	30.72±10.37	60.67±16.00	80.87±13.83
TED vs ND	4.97±1.85	26.21±4.26*	46.27±5.01*	61.13±2.92*
	6.35±3.01	15.19±7.17	22.97±8.91	37.87±8.23
TED vs TEC	4.97±1.85*	26.21±4.26	46.27±5.01	61.13±2.92*
	9.00±4.01	21.40±14.97	34.68±11.93	44.64±3.50
TPD vs TPC	16.55±3.44*	25.24±4.62	43.48±10.80*	58.54±32.61*
	12.31±3.28	32.89±12.70	63.22±14.45	80.85±4.92
TPC vs SD	12.31±3.28	32.89±12.70	63.22±14.45	80.85±4.92
	11.34±6.76	30.72±10.37	60.67±16.00	80.87±13.83
TPC vs ND	12.31±3.28*	32.89±12.70*	63.22±14.45*	80.85±4.92*
	6.35±3.01	15.19±7.17	22.97±8.91	37.87±8.23
TPC vs SC	12.31±3.28	32.89±12.70	63.22±14.45	80.85±4.92
	14.31±3.38	36.07±9.56	66.68±5.01	85.40±3.24
TPC vs NC	12.31±3.28	32.89±12.70	63.22±14.45	80.85±4.92
	9.59±3.62	27.89±9.67	51.83±18.42	75.17±5.70
TED vs SC	4.97±1.85*	26.21±4.26*	46.27±5.01*	61.13±2.92*
	14.31±3.38	36.07±9.56	66.68±5.01	85.40±3.24
TED vs NC	4.97±1.85*	26.21±4.26	46.27±5.01	61.13±2.92*
	9.59±3.62	27.89±9.67	51.83±18.42	75.17±5.70
TEC vs SC	9.00±4.01*	21.40±14.97	34.68±11.93*	44.64±3.50*
	14.31±3.38	36.07±9.56	66.68±5.01	85.40±3.24
TEC vs NC	9.00±4.01	21.40±14.97	34.68±11.93	44.64±3.50*
	9.59±3.62	27.89±9.67	51.83±18.42	75.17±5.70
SD vs ND	11.34±6.76	30.72±10.37*	60.67±16.00*	80.87±13.83*
	6.35±3.01	15.19±7.17	22.97±8.91	37.87±8.23
SD vs SC	11.34±6.76	30.72±10.37	60.67±16.00	80.87±13.83
	14.31±3.38	36.07±9.56	66.68±5.01	85.40±3.24
SD vs NC	11.34±6.76	30.72±10.37	60.67±16.00	80.87±13.83
	9.59±3.62	27.89±9.67	51.83±18.42	75.17±5.70
ND vs NC	6.35±3.01	15.19±7.17*	22.97±8.91*	37.87±8.23*
	9.59±3.62	27.89±9.67	51.83±18.42	75.17±5.70
NC vs SC	9.59±3.62*	27.89±9.67	51.83±18.42	75.17±5.70*
	14.31±3.38	36.07±9.56	66.68±5.01	85.40±3.24

*The comparisons of mean group wound contraction rates revealed that some differences were of statistical significance (P≥0.05) such are indicated with asterisk**

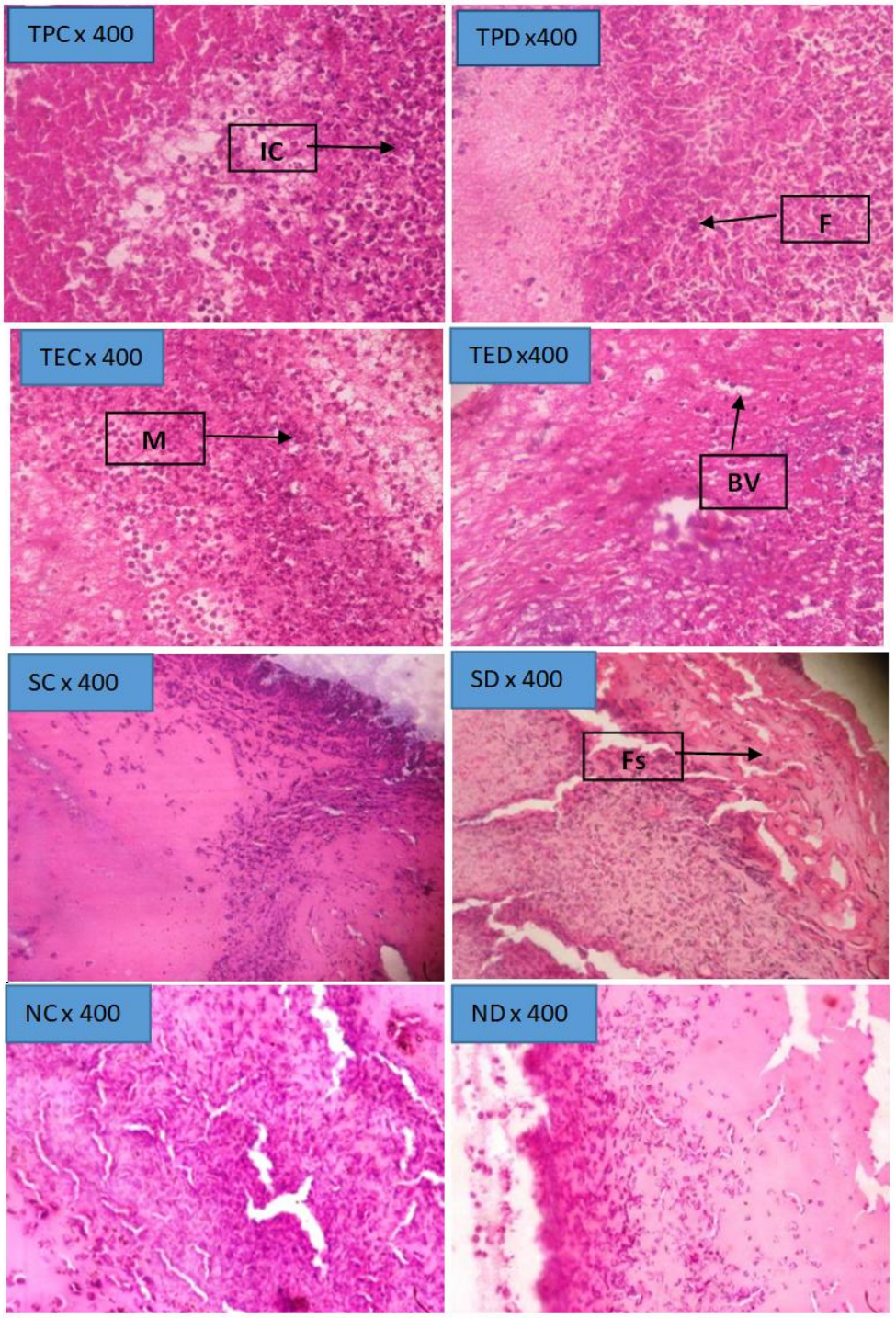

Plate 1. Granulation tissue at Day 3 (H & E).

TPC (Tobacco powder control), TPD (Tobacco powder diabetic), TEC (Tobacco extract control), TED (Tobacco extract diabetic), SC (Sofratulle control), SD (Sofratulle diabetic), NC (Normal Saline control), ND (Normal Saline diabetic), BV (Blood vessel), IC (Inflammatory cells), F (Fibroblast), Fs (Fibrillary structures) and M (macrophages)

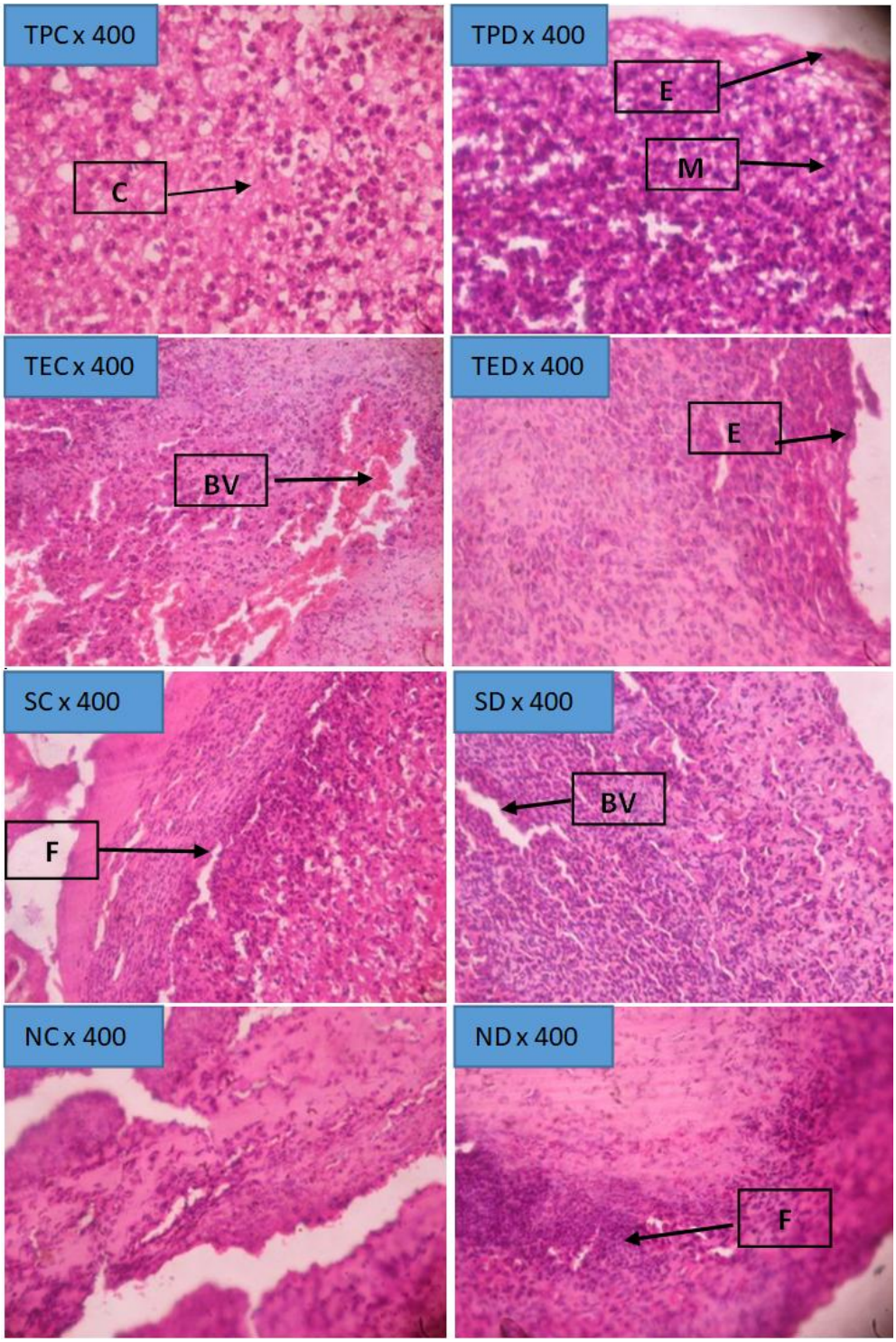

Plate 2. Granulation tissue at Day 6 (H & E).

TPC (Tobacco powder control), TPD (Tobacco powder diabetic), TEC (Tobacco extract control), TED (Tobacco extract diabetic), SC (Sofratulle control), SD (Sofratulle diabetic), NC (Normal Saline control), ND (Normal Saline diabetic), BV (Blood vessel), IC (Inflammatory cells), E (stratified squamous epithelium), F (Fibroblast), Fs (Fibrillary structures) and M (macrophages)

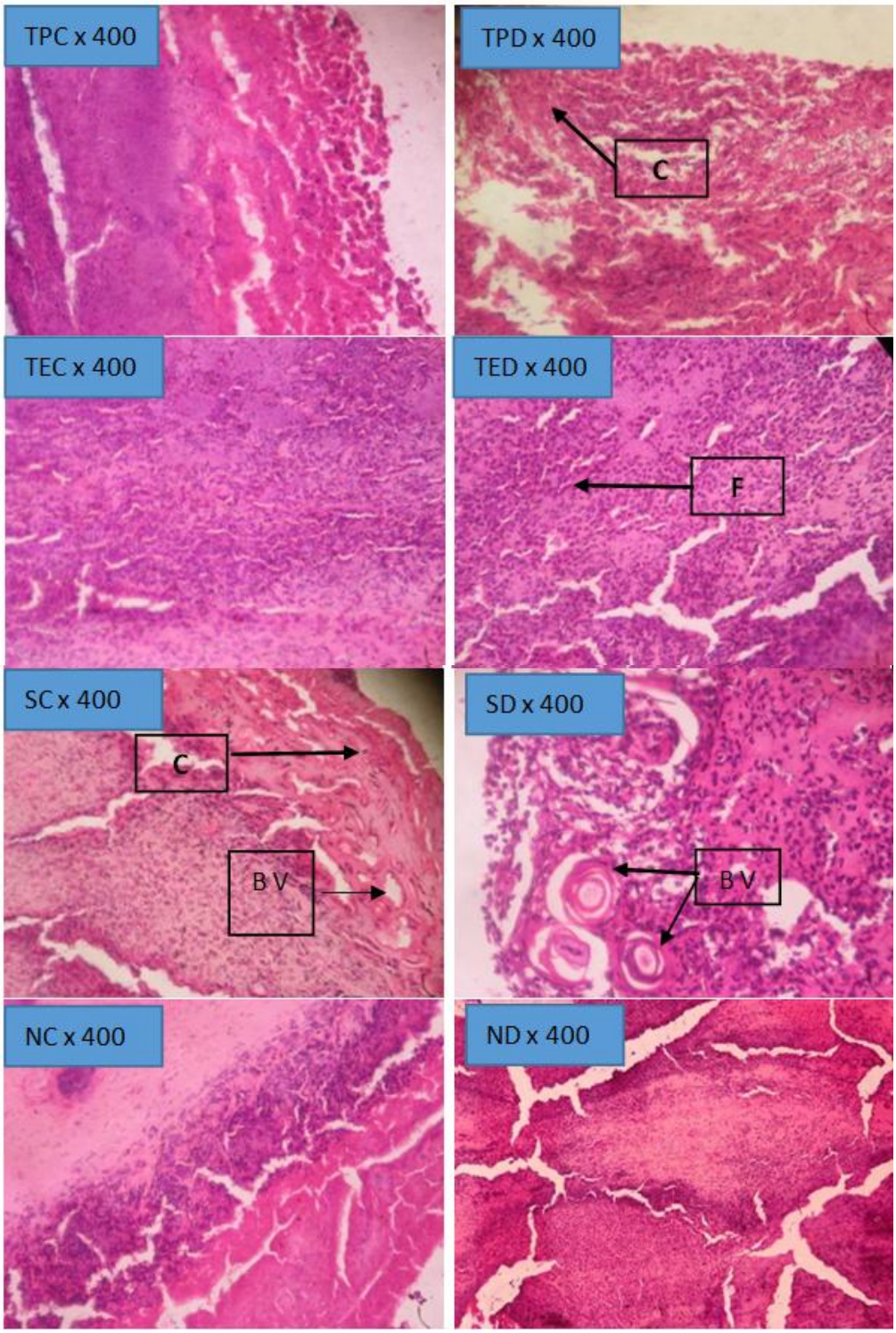

Plate 3. Granulation tissue at Day 9 (H & E).

TPC (Tobacco powder control), TPD (Tobacco powder diabetic), TEC (Tobacco extract control), TED (Tobacco extract diabetic), SC (Sofratulle control), SD (Sofratulle diabetic), NC (Normal Saline control), ND (Normal Saline diabetic), BV(blood vessel) C (Collagen) and F (Fibroblast)

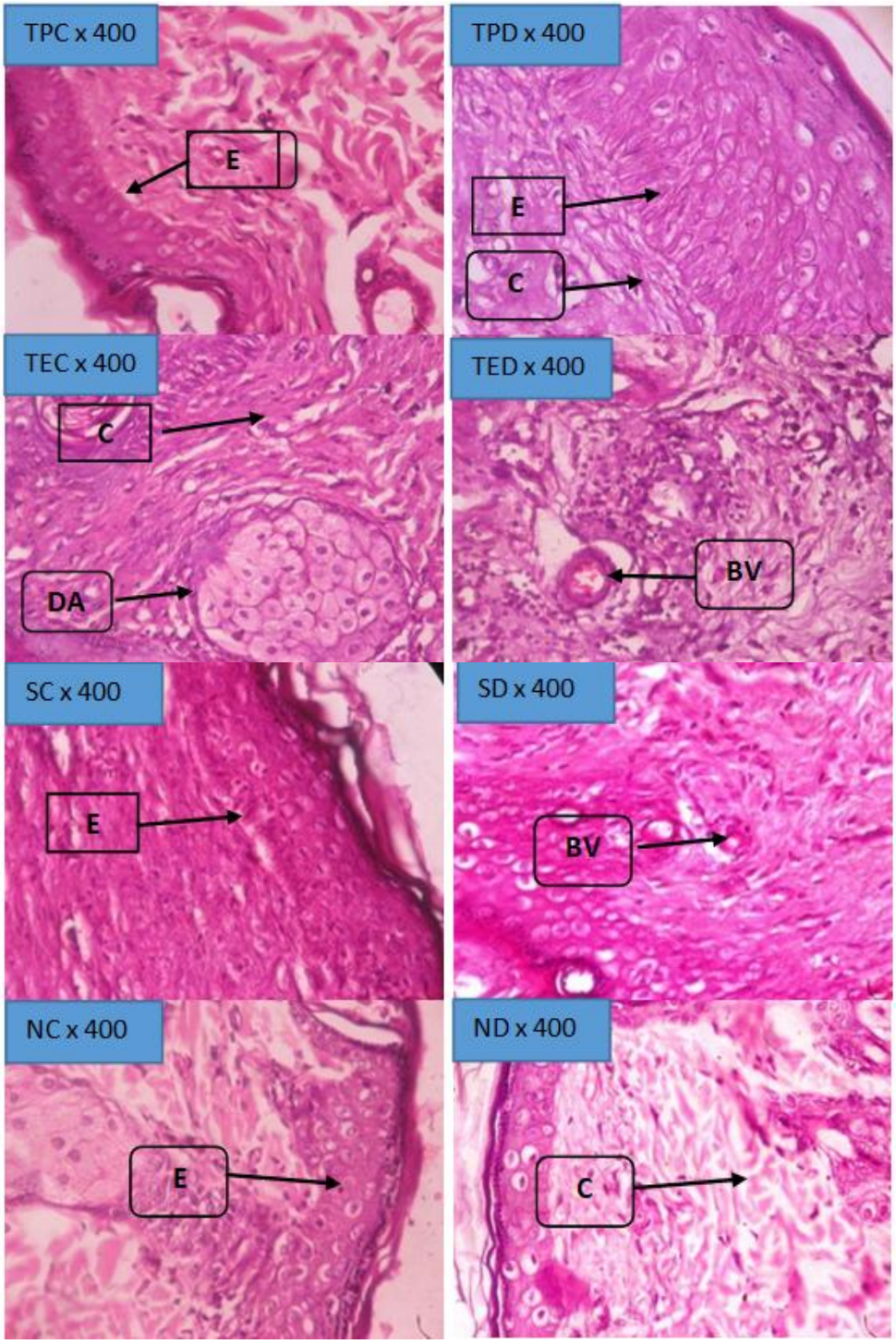

Plate 4. Sections from the wound scars (H & E)

TPC (Tobacco powder control), TPD (Tobacco powder diabetic), TEC (Tobacco extract control), TED (Tobacco extract diabetic), SC (Sofratulle control), SD (Sofratulle diabetic), NC (Normal Saline control), ND (Normal Saline diabetic), BV (Blood vessels), C (Collagen), DA (Dermal appendages), E (stratified squamous epithelium) and F (Fibroblast)

4. DISCUSSION

The results of the phytochemical analyses of *Nicotiana tabacum* of both the powder and the ethanolic extracts were similar to those obtained by other studies [4,6] thus the grade of the plant used in this study was of comparable standard. One of the symptomatology of diabetes mellitus is weight loss; progressive weight losses were observed in all the diabetic groups coupled with the sustained elevated mean blood sugar levels (hyperglycemia) in same groups. This thus confirmed that wound healing processes in this study occurred in a diabetic milieu (setting).

Diabetes is known to retard wound healing, but the tobacco powder diabetic group had the highest mean wound contraction rate on day 3 even than its control. However, at subsequent periods i.e. day 6,9,12 and 18; this was not observed. Thus tobacco powder did not accelerate healing of diabetic wound as it would have been suggested by the day3 result. Another noteworthy observation was the mean wound contraction rates of the diabetic tobacco ethanolic extract group being higher than those of the control (TEC) for most of the duration of the study (day 6,9,12,15 &18); this was even significant on day 12. From the foregoing, it thus appeared that the ethanolic extract of *Nicotiana tabacum* may be beneficial in the management of diabetic wounds. The tobacco powder control rates compared favourably with those of sofratulle groups (control and diabetic) with no significant difference. Sofratulle is a standard wound dressing material and synthetic while tobacco is a naturally occurring plant, the preparation of its crude powder requires no special skill and this can easily be taught and acquired.

Diabetic wounds are often chronic and one the challenges in management is the cost of sofratulle. Though, the cost of sofratulle is not exorbitant, when the economic empowerment of most of the patients with diabetic wounds / ulcers is factored into the cost of management (and of course it should be) then the affordability and accessibility of sofratulle by most of our patients becomes a major factor to reckon with. Globally, tobacco is largely consumed (smoke or sniff) for recreational purposes with well and widely documented major health hazards to the extent that its consumption is being discouraged. Thus the comparability of the wound contraction rates of tobacco powder with sofratulle in rats if translatable to human diabetic wound management would be a major feat and also a beneficial effect of *N. tabacum* to human being. However, in non-diabetic wounds, sofratulle appeared to promote wound healing better than tobacco ethanolic extract as evidenced by significantly higher wound contraction rates on day 3,9 and 12.

The mean rates for the tobacco powder diabetic were significantly lower than those of the control, thus it would be appropriate to conclude that the wound healing potency of *N. tabacum* powder was retarded by diabetes. Both the TPD and TED groups had similar mean wound contraction rates thus it might be inferred that *N. tabacum* could serve as wound dressing material either in powdery or extract formulation. This becomes a pertinent point when one considers the fact that it is easier to prepare the powder than the ethanolic extract as the former is less technically demanding.

The mean wound contraction rates of the tobacco powder diabetic group were significantly higher than those of the normal saline diabetic group throughout the duration of the study. Even as of day 12, the mean rate for TPD was less than 60%, thus it would not be inappropriate to state that normal saline is a poor wound dressing material especially in a setting of diabetes. Consequently, normal saline should only be used to irrigate wounds and not as a wound dressing material especially in diabetics.

Tobacco smoking is said to impede the inflammatory phase of wound healing by reducing cellular chemotaxis, migration and oxidative bacterial phagocytosis [9-12]. It also retards the proliferative phase of wound healing by reducing fibroblast proliferation and migration with consequent diminished collagen synthesis [13-15]. Nicotine, the major compound of tobacco is a potent vasoconstrictor and is rapidly absorbed during smoking this leads to reduced tissue perfusion (especially cutaneous) and this ultimately results in tissue hypoxia [16-17]. The fundamental pathway by which tobacco smoke affects the two previously mentioned phases of wound healing has been attributed to tissue hypoxia [18,19]. Tobacco smoke due to its carbon monoxide and hydrogen cyanide content has been associated with reduced proliferation of white blood cells (leukocytes) and fibroblasts [20,21]. In this study, it was the non-smoke tobacco (powder and ethanolic extract) that was used this might be the reason why reduced leukocyte infiltration and fibroblasts was not observed.

Epidermal growth factor (EGF) is crucial to the stimulation of epidermal regeneration by keratinocytes. The EGF, which is normally secreted by platelets, fibroblasts and macrophages subsequently stimulates keratinocyte migration and proliferation. Some human studies carried out in smokers have documented reduced levels of EGF [9,22,23]. This could explain the delayed reepithelization that was observed in all the tobacco groups but slightly more pronounced in the diabetic groups (TPD and TED) in our study.

Intense leukocyte infiltrates were observed in the granulation tissues of all the tobacco groups on day 3 and 6. This cellular infiltration was more pronounced in the two diabetic groups. Similar profuse leukocyte infiltration was observed in the injured nasal mucosal of rat exposed to tobacco smoke [24]. Thus one of the mechanisms by which *N. tabacum* retards wound healing is via increased severity and prolongation of the inflammatory phase healing.

Chronic diabetic foot ulcers are due to peripheral angiopathy and neuropathy. A 2005 study estimated prevalence of diabetes foot ulcer amongst diabetics in the United State of America to be 1 in4 and that 15% of this subset of patients will need amputation [25] with diabetic foot ulcer accounting for 25-50% of the costs related to diabetic care [26]. This statistic may be higher or lower in medium and low economy countries. Thus the management of chronic wounds of which diabetic foot ulcer is very prominent poses a major social and economic burden. Documented surgical complications of tobacco smoke exposure include delayed wound healing, delayed bone union, anastomotic leakage, wound dehiscence, respiratory complications and increased intensive care admissions [27-29]. Thus wounds in diabetics that are also tobacco smokers are very challenging and may be difficult to manage as it invariably entails multi-disciplinary and multi-specialty approaches.

Low concentrations of topically applied nicotine have been found to promote angiogenesis with consequent improved wound healing. In a study in which excised wounds of mice were dressed with silicon sheet impregnated with nicotine. The degree of wound contraction, epithelization and angiogenesis were greater than those dressed with phosphate buffer saline but of same magnitude with those dressed with basic fibroblast growth factor [30].

In this study, histology of the sections from the scars showed well demarcated stratified squamous epithelium, subcutaneous layer rich in collagen fibres, blood vessels and skin appendages in all the groups. Though, the sofratulle groups had higher wound contraction rates than the tobacco groups; this observation could infer that that the topical application of *N. tabacum* powder and extract produced scars comparable with those of sofratulle. Thus topical application of *N. tabacum* promotes wound healing. Some studies demonstrated that nicotine which is the main constituent of *N. tabacum* when topically applied in genetically diabetic mice promoted angiogenesis both *in vivo* and *in vitro*, thus in essence promoting wound healing [31,32]. Nicotine is known to alter release of growth factors such as basic fibroblast growth factor (bFGF) and transforming growth factor beta (TGF-β). Also nicotine has been demonstrated to up regulate the expression of vascular endothelial growth factor (VEGF) in endothelial cells [33-35]. These cytokines are responsible for cellular release and migration; fibrin and collagen synthesis; and neovascularization which are essentially the stages/ phases of wound healing. Thus the stimulation of the expression of bFGF, TGF-β and VEGF by nicotine may the pathway by which topically applied *N. tabacum* promotes wound healing.

Other clinical entities in which applications of nicotine have been investigated include tobacco cessation, neurological disorders, recurrent aphthous ulcers, ulcerative colitis, pemphigus and pyoderma gangrenosum [36-39].

5. CONCLUSION

The results of this study showed that the powder and ethanolic extract of *Nicotiana tabacum* leaves possess the potency of accelerating wound healing in wistar rats when applied topically to cutaneous wound. The non-recreational but clinical utilizations of *N. tabacum* (tobacco) had been and still being widely investigated and as the research findings become more promising and translated to human health management; the prevalence of multi systemic diseases associated with tobacco usage and its attendant problems will start to decline

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animals were handled in accordance to the guidelines as prescribed by the ethical conduct of animal research of the University of Ibadan. Also, the principles of laboratory animal care as contained in the 8th edition (2011) of the Guide for the Care and Use of Laboratory Animals by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals were observed. (National Academies Press (US); 2011. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050/doi: 10.17226/12910).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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