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Effects of prolonged oral administration of sildenafil citrate on wound healing of diabetic rats

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ABSTRACT

Wound healing is a major problem in diabetic patient. Sildenafil citrate was earlier reported as a pro-angiogenic agent found to stimulate angiogenesis. We decided to investigate if oral administrations of sildenafil citrate in type I diabetic rats will enhance wound healing. The study was performed using fourty two rats (42) weighing between 139-225 grams. The rats were grouped A-G, six (6) rats per group. Wound area of 1.5 by 1.5 cm^2 was created at the dorsal surface of each rat under sedation with ketamine and local anaesthetic (lignocaine). Type I diabetes was induced using Alloxan monohydrate at dose rate of 130 mg/kg. Sildenafil citrate was administered at a dose rate of 50 mg/kg orally daily for 21 days and 10 international units of insulin was administered intraperitoneally to the control group once. Blood glucose and platelet count were significantly ($p<0.05$) decrease compared to pre-diabetic period. The wound size contraction did not significantly ($p>0.05$) decrease as compared to day 3 in diabetic rats. We concluded that oral administration of sildenafil citrate at dose rate of 50 mg/kg did not significantly enhance wound healing in diabetic rats.

Keywords: Sildenafil citrate, diabetic, wound healing, rats

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INTRODUCTION

Sildenafil citrate is a specific inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterases 5 (PDE5)[1]. It was earlier used as an antianginal drug and also used to treat pulmonary hypertension[2], but due to an unexpected side effect caused erection in males. Sildenafil citrate was reported to increase neovascularization on the chick chorioallantoic membrane, increased endothelial cells proliferation and migration through inhibition of PDE5[2]; it was also reported to enhanced wound healing process in normal rat[3-5] but was not reported in diabetic rat model. In fact, sildenafil citrate application was found to decrease significantly the size of the skin wound in a dose independent manner during normal healing process[6].

Diabetes is a multi systemic disorder that affects wound healing process. It has been implicated during acute and chronic wound healing process[7]. But, Type 2 diabetes (non-insulin dependent) continues to increase in incidence and is more prevalence in older age patients[8], in which age-related skin changes already negatively impact on the wound healing process. Wound healing which is dynamic and complex process that involves well coordinated and highly regulated series of event including inflammation, tissue formation revascularization and remodeling[9, 10]. This orderly process is impaired in certain pathologic condition, including diabetes; making diabetic wounds a great problem to be healed[11]. On the other hand, the severity of diabetic complications can also be related to the magnitude of sub-products called advanced glycosylation products, which are heterogeneous group of structures which significantly increase in sera and tissues of aged and diabetic patients[12, 13]. [14]reported that sildenafil citrate enhance proliferation of new capillaries by its vasorelaxant effect on wound healing. Also another independent group[4] reported that sildenafil citrate is used as supportive factor in wound healing process.

However, there has been no study on the oral or topical use of sildenafil in different phases of wound healing in diabetic model. In wound healing, the acute inflammatory phase is characterized by a neutrophil infiltration, replaced by mononuclear cells later. The proliferative phase is characterized by the presence of mononuclear cells infiltration, proliferation of fibroblast keratinocytes, granulation tissue formation with angiogenesis[15], with the proliferation of endothelial cells and deposition of extracellular matrix molecules. The final phase represents the maturation of neo-formed tissue[16]. Hence, understanding the process at sub-molecular level may lead to the design of better treatment regimen[17].

To gain further understanding on the role of cGMP beyond erectile dysfunction and pulmonary hypertension, since in diabetic animals the wound healing process is attenuated.

We therefore hypothesized whether oral administration of sildenafil citrate will enhance wound healing in a diabetic rat model.

MATERIALS AND METHODS

A total of forty two (42) Wister albino rats (10-12 weeks) of both sexes were obtained from the Sanda Kyarimi Zoo Maiduguri. The rats were housed and acclimatized in an individual cage for three (3) weeks prior to the experiment. The rats were fed standard pellets (Vital feed growers) and water ad-libidum. The rats were then weighed and randomly divided into seven (7) treatment groups with each group having six (6) rats. The animals were handled according to the International Guiding Principles for Biomedical Research Council for International Organizations of Medical Sciences [18].

Diabetes was induced by a single intra-peritoneal injection of prepared alloxan mono-hydrate at dose rate of 130 mg/kg as earlier described by [19] with slight modifications.

Diabetes was confirmed three (3) days after administration by measuring the fasting blood glucose concentration using glucometer. Only rats with blood glucose level of 180 mg/dl and above were used for the experiment.

Surgical procedure, the fur was shaved using a scissors and electric clipper, the back of the rats were shaved under local anesthetic (lignocaine), a square shaped wound measuring 1.5cm × 1.5cm was created on the back of the rats by using a scalpel blade and scissors.

As shown above group A, B, C and D are diabetic rats. So wound was created in group A, B, C, D, E and F. Then, oral sildenafil citrate was administered at a dose rate of 50 mg/kg daily to group A, B, E and G, and 10 international unit of insulin was administered to group B and C by intra peritoneal route (I.P) once.

Wound dressing was performed using a mild antiseptic solution (liquid Dettol) on all the groups with wound (i.e. A, B, C, D, E and F) on day 3 only.

Materials for wound creation

Materials used includes; scalpel blade, rat tooth forceps, metallic divider, ball point pen, transparent plastic ruler and hard plastic material (ruler) having a suitable handle with a surface of 1.5cm x 1.5cm.

Wound Creation

Rats were anaesthetized intraperitoneally using 50 mg/kg Ketamine hydrochloride (RotexMedica, Tritau, Germany) and 5 mg/kg Sedazine® (Fort Dodge Animal Health, Iowa, U.S.A.) with a combined dose obtained following a pilot study using 1ml syringe. Onset of anaesthesia ranged between 4-8 minutes. After induction of anaesthesia, the hair on the dorsal region of the rat was shaved with an electric clipper and scissors, the outline of the area for wound creation was done by diligently pressing the plastic material against the skin of the rat

and the outline made by the marker. The area was sterilized with 70 % alcohol and full thickness skin was excised along this outline. This procedure was repeated on the dorsum of each rat at approximately 1.5×1.5 centimetre square. Haemorrhage was controlled by dabbing using sterilized gauze on the wound area. The wound was left undress with no local or systemic antibiotics.

Measurement of Wound Contraction

The measurement of the wound size is carried out using a well label measuring ruler, the ruler were placed directly on the wound for both the length and breadth the readings were taken from 1cm graduation up to the terminal point of the wound. Photographs were taken after every 48 hours for 21days.

Determination of Platelet count

Blood was collected through the tail vein, at day zero for determination of platelet as previously described by[20], then at weekly interval until three weeks.

Materials

Neuber counting chamber, thomma red cell pipette, red blood cell diluents (1% ammonium oxalate), blood sample, scissors, 70% alcohol, cotton wool and Olympus electric microscope.

Methods of Determination of platelets count.

The blood drawn from the tail vein of rats into the RBC pipette up to 1.0 mark, the tip of the pipette was cleaned and thereafter the platelets diluting fluid was drawn up to the 101 mark, this gave the dilution factor of 100. The content of the pipette was mixed thoroughly by rolling in the palm and thereafter the chamber was charge with the fluid and then counted using ×40 objective light microscopes.

Calculations:

The procedure for counting platelets is the same as that of RBC. Let the number of cells counted in 80 Small square = n

$$\text{Area of Chamber} = \frac{1}{10}\ \text{mm}$$

$$\text{Area of Small Squares} = \frac{1}{400}\ \text{mm}^2$$

$$\text{Volume} = \frac{1}{400} \times \frac{80}{1} \times \frac{1}{10}$$

Dilution factor = 100

Statistically analysis

All data were expressed as Mean ± SEM using one way analysis of variance (ANOVA), Computer software SPSS version 20 was used for the analysis and $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effects of sildenafil citrate on mean blood glucose of diabetic rats

The result of effects of sildenafil citrate on blood glucose of diabetic rats was shown (Table I). Sildenafil citrate was shown to decrease blood glucose (Table I) to normal values when compare to pre-diabetic rats, the decrease was observed at 21 days treated with sildenafil (Table I). Moreover, sildenafil citrate (Group A; Table I) seems to have less activity on the mean blood glucose level of diabetic rats when compare with positive control (group C). In overall the mean blood glucose was significantly ($p<0.05$) decreased at 21 day in (Group A; Table I) when compared to the pre--diabetic rats.

Table 1: Effects of sildenafil citrate on blood glucose (mg/dl) of diabetic rats

Group	Days of Treatment				
	Prediabetic	0	7	14	21
A	96.00±3.04	301.25±45.76*	492.33±53.30	451.33±98.10	151.67±13.62**
B	86.6±4.23	189.00±77.46*	298.67±150.95	327.33±124.29	186.33±71.05
C	97.17±6.23	403.25±96.95*	423.33±149.16	423.33±176.67	218.67±60.41
D	83.00±3.13	368.20±83.17*	309.25±63.39	263.50±106.57	215.25±34.25

*Mean difference is significant at $p < 0.05$ from pre-diabetic value(s). ** Mean statistical significance between days of treatment.

Effects of sildenafil citrate on wound contraction size in diabetic rats

Table II is the result of effects of sildenafil citrate on wound contraction size of diabetic rats, the diabetic wound (Group A; Table II), significantly ($p<0.05$) contracted wound size at day 19 and the percentage contraction in wound size was 98.3% in sildenafil (group A; Table II) as against 99.6% in positive control (group C; Table I) at the same day. The percentage contracted wound size in (Group B; Table II) was 94.0%. However, the percentage wound size contraction in negative control (Group F; Table II) was 87.3%. The wound size contraction in diabetic rats (Group E; Table II) at 11, 13 and 15 days were 48.3%, 70.4% and 86.8% respectively.

The contracted wound size in diabetic rats in this study appears to have no significant difference (Table II; figure 1) pictures insert, which also suggest that sildenafil citrate has minimal effects on wound healing process in diabetic rats via oral route.

Table II: Effects of Sildenafil citrate on wound contraction size of diabetic rats (cm^2).

	Treatment Days										
Group	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**	**21**	
A	2.99±0.792	2.24±0.9	2.04±0.6	2.27±0.7	1.77±0.9	1.33±0.8	0.65±0.4	0.42±0.3	0.30±0.2*		0.05±0.0
B	2.36±0.386	3.17±0.6	3.39±0.8	2.97±1.0	2.19±1.2	1.01±0.1	0.54±0.4	0.16±0.1	0.13±0.1		0.05±0.0
C	2.71±0.03	3.00±0.4	2.73±0.5	2.28±0.5	1.56±0.7	0.93±0.5	0.32±0.2*	0.0±0.0*	0.01±0.0*		0.00±0.0*
D	1.66±0.1	2.31±0.1*	2.45±0.3*	2.44±0.1*	1.76±0.1	0.88±0.1*	0.37±0.0*	0.15±01*	0.03±0.0*		0.00±0.0*
E	2.13±0.15	2.11±0.2	2.02±0.2	1.91±0.2*	1.10±0.1*	0.63±0.1*	0.28±0.0*	0.15±.0*	0.06±0.0*		0.01±0.0*
F	2.21±0.067	1.96±0.2	1.65±0.2	1.67±0.2	1.32±0.3	0.81±0.2*	0.50±0.2*	0.4±0.3*	0.28±0.2*		0.03±0.0*

* Mean difference is significant at $p<0.05$ from day 3.

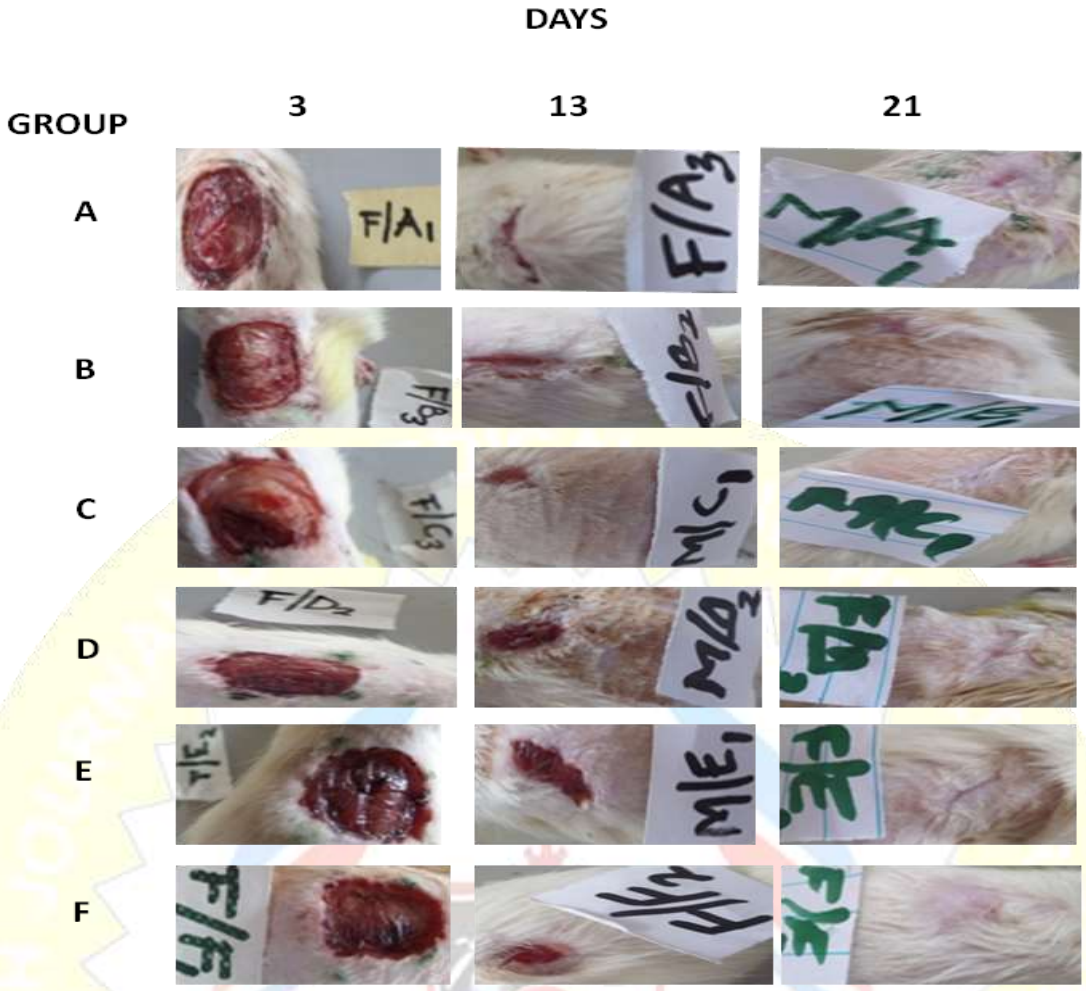

Figure 1: The photo insert showing different phases of wound size contraction in diabetic rats at day 3, 13 and 21 respectively, following oral administration of sildenafil citrate.

Effects of sildenafil citrate on mean platelets count of diabetic rats

Table III is the effects of sildenafil citrate on mean platelet count of diabetic rats. The mean platelet significantly decreased ($p<0.05$) when compare with pre-diabetic rats. The decreased seems to be more in (Group F and G, Table III) especially at day 7, 14 and 21 respectively.

Table III: Effects of Sildenafil citrate on mean platelet counts ($\times 10^6$ /L) of diabetic rats for 21 days.

	Treatment days				
Group	**Pre-diabetic**	**0**	**7**	**14**	**21**
A	407.5±71.7	262.6±5.4	111.6±18.*	220±50	188.3±14
B	313.3±26.5	233.3±27	155±18.0*	225±30.5	172±9.0*
C	357.5±52.7	480±84.5	201.6±4.4	213±35	151.6±32
D	330.8±43.9	299.6±69.	182.5±11	176±11	151±4.2

* Mean difference is significant at $p<0.05$ from day 0.

Group identification**:**

Definition of treatment groups

A= Diabetes + wound + Sildenafil.

B= Diabetes + wound + Sildenafil + insulin.

C= Diabetes + wound + insulin.

D= Diabetes + wound

E= Wound + Sildenafil.

F= Wound + injection water.

G= Sildenafil citrate alone.

DISCUSSION

Sildenafil citrate was earlier reported as pro-angiogenic agent that stimulates angiogenesis through a protein kinase G/MAPK pathway[2], the study was demonstrated in endothelial cells (using micro molar concentration of sildenafil) and found to increase cell growth, migration and capillaries like formation *in vitro* and *in vivo*. In this study we carried out an investigation if sildenafil citrate can have similar effects on wound healing of diabetic rats which is a frequent and severe problem in patient with diabetes [21].

From our results sildenafil citrate seems to decrease the mean blood glucose of the diabetic rats, with high blood sugar levels if wound healing was delayed. The high blood sugar level tend to stiffen the arteries and cause narrowing of the blood vessels, which slows down post-surgical and chronic wound healing process in rats. This leads to the reduction of vital blood flow and oxygen directed toward the affected area(s) which the body uses during the natural healing process. Uncontrolled or elevated blood sugar levels also restrict the function of red blood cells, which normally carry nutrients to the damaged tissue. When red blood cells are prevented from carrying the maximum amount of blood and healing oxygen, the white blood cells which fight against the infection are also slowed down. Another important role has also been attributed to the intrinsic factors and the biology of chronic wound healing in diabetes[21].

It has been postulated that hyperglycemia itself has a deleterious effect on the wound healing through the formation of advanced glycation-end products which induce the production of inflammatory molecules that could interfere with collagen synthesis. Our findings was in disagreement with[6], where they used different gel (%) preparations of sildenafil-carbopol topically applied directly to the wound area in normal rats, that means sufficient amount of the drug could have been absorbed and reached circulation, also more surface (cm^2) area of wound created in our study, could be an additional factors attributing to less healing effects of sildenafil citrate in our study.

The fact that oral administration of sildenafil citrate in diabetic rats did not show any significant difference in wound size contractions is not surprising, the earlier report by[2], the experiment was carried out on cell lines, probably there is need to further optimize dosage in our experiment. The 50 mg/kg used in our study may be is not sufficient enough to exert

effects on wound size contractions, in addition from oral route, the absorbed quantity of drug could not be sufficient to reach circulation to produce desirable effects. Some drugs may be inactivated which could further interfere with normal drug absorption especially for drug with first pass effects. The slight percent decreased in wound size contraction noticed in diabetic could also be attributed to decrease number of platelets in circulations. Since wound healing is stage process which involves activity of leucocytes and platelet, for this reason sufficient number of platelets in circulation is vital to guide regenerating cells to the area of healing[22]. When tissue is wounded, blood comes in contact with collagen triggering to begin secreting inflammatory factors, platelet also express glycoprotein on their membranes surface which allow them to stick together to form an aggregates and mass of tissues [19]. Platelets have also been reported to play role in vascular homeostasis[23]. Platelet receptor could interacts with collagen receptor and von Willebrand factors among others that may capture the platelet to induce signal activation and participate in formation of fibrin and clotting complex which could also be of benefit in healing process. Arguably, in our findings we concluded, beyond doubt for the first time we reported that oral administration of sildenafil citrate at dose rate of 50 mg/kg did not significantly enhance wound healing in diabetic rats. But continue search through research at cellular and molecular level could put to beside modern and more effective therapy in management of diabetic wound.

Therefore, with the ongoing research and investigations in the field of medicine and modern technologies could eventually become more useful to people with diabetic wound in the near future. One of the major steps is the integration of these resources in the coordinated effort to make the technology developed at the bench available to the patients at the bedside. Moreover, it is this synergistic therapy with further studies at cellular and molecular level that could help to eliminate amputations in patients with diabetes.

CONCLUSION

Conclusively, sildenafil citrate administered orally did not significantly decrease wound size contraction in diabetic rats. We therefore recommend other routes of administration, such as topical and systemic to be evaluated for the same effects.

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REFERENCES

1. Baykan H, Ozyazgan I and Selcuk CT . (2013). Effect of sildenafil citrate in nicotine-induced ischemia: experimental study using a rat model. Can J PlastSurg; 21(4):217-

20.

2. Pyriochou A, Zhou Z, Koika V, Petrou C, Cordopatis P, Sessa W and Papapetropoulos A. (2007).The phosphodiesterase 5 inhibitor sildenafil stimulates angiogenesis through a protein kinase G/MAPK pathway. J Cell Physiol.211: 197-204.
3. Brueckner CS, Becker MO, Kroencke T, Huscher D, Scherer HU, Worm M, Burmester G and Riemekasten G (2010). Effect of sildenafil on digital ulcers I systemic sclerosis: analysis from a single centre pilot study. Ann Rheum Dis 69: 1475-1478.
4. Derici H, Kamer E, Unalp HR, Diniz G, Bozdag AD, Tansug T, Ortac R and Erbil Y. (2010). Effect of sildenafil on wound healing: An experimental study. *Langenbecks Arch Surg*395: 713-8.
5. Farsaie S, Khalili H, Karimzadeh I and Dashti-Khavidaki S. (2012). An old drug for a new application: potential benefits of sildenafil in wound healing. J Pharm PharmacSci 15: 483-498.
6. Gursoy K, Melike O, Yuksel K, M GurhanUlusoy, Ugur K, Duygu K, R. NeslihanGursoy, Ozge C, Elmas O and VildanFidanci. (2014). Effect of topically applied sildenafil citrate on wound healing: Experimental study. Boson J Basic Sci;14(3):125-131.
7. Mulder GD. (1998). Physiology of wound healing. In leaper D and harding K (Eds) wounds: Biology and management. New york NY, Oxford University Press.
8. Cruickshank JK. (1997). Epidemiology and geography of non-insulin G, Eds. Blackwell, Oxfort, U.K. P.17-29.
9. Hamed S, Ullmann Y, Masoud M, Hellou E, Khamaysi Z and Toet L. (2010). topical erythropoietin promotes wound repair in diabetic rats. J Invest. Dermatol, 130: 287-294.
10. Toker S, Aulcan S, Cayel MK, Olgun EG, Erbilen E and Ozag Y (2009). Topical atorvastatin in the treatment of diabetic wounds. Am.J.Med.Sci, 338:201-204.
11. Valls MD, Crostein BN and Montestinos MC. (2009). Adenosine receptor agonists for promotion of dermal wound healing. Biochem Pharmacol, 77:1177-1224.
12. Ptak W, Klimek M, Bryniarski K, Ptak M and Majcher P. (1998). Macrophages function on alloxan diabetic mice: expression of adhesion molecules, generation of monokines and oxygen and NO radicals. Clin exp. Immunoles. 114:13-18.
13. Vlassara , H. (1992) . Cell-mediated Interactions of Advanced Glycosylation end products and the vascular wall. In: Hyperglycemia, diabetes and vascular disease . Oxford University Press, New York, Oxford.

14. Tas A, Atasoy N, Ozbek H, Asian L, Yuksel H, Ceylan E and Dagoglu G. (2003) .The effects of sildenafil citrate (Viagra) in the early phase of healing process in open wounds in dogs. Acta Vet BRNO 72:273-277.
15. Singer AJ and Clark RAF. (1999). Mechanism of disease. cutaneous wound healing. N.EngJ.Med, 341:738-746.
16. Loots MA M, Lamme J, Zeegelarr JR, Mekkes JD, Bos JD and Middlekoop E. (1998). Difference in acute infiltrate and extracellular matrix of chronic Diabetes and venous ulcers versus acute wounds. J. Invest. Dermatol, 111:850-857.
17. Rendell MS, Johnson ML, Smith D, Finney D, Capp C, Lammers R, and Lancaster S. (2002). Skin blood flow response in the rat model of wound healing: expression of vasoactive factors. *J Surg Res* 107: 18-26.
18. Council for International Organization of Medical Sciences, C.I.O.M.O, (1985). International Guiding Principles for Biomedical Research Involving Animals. 1211, Geneva 27, Switzerland, c/o W.H.O.
19. Ajayi EIO, Popoola G and Ojediran E. (2016). Wound healing potential of *Nauclealatifolia* and *Monihotesculentum* leaf extract in Type I diabetic rats. Afr. J. Traditional complement. Alternative medicine. 1:1-5.
20. Coles EH. (1986). Veterinary clinical pathology 14th ed. W.B Sanders Company Philadelphia Pp. 457.
21. Tsourdi E, Barthel A, Rietzsch H, Reichel A and Bornstein S.R (2013). Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. Biomedical research international. Volume 2013, article ID 385641, 6 pages.
22. Khiste SV and Tari RN. (2013). Platelet-rich fibrin as a biofuel for tissue regeneration. Hindawi Publishing Corporation. Volume 2013, Article ID 627367, 6 pages.
23. Golebiewska EM and Poole AW. (2015). Platelet secretion from haemostasis to wound healing and beyond. 29: 153-162.