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The Influence of Leaves Extracts of *Azadirachta Indica* and *Mangifera Indica* on High Blood Glucose Level and Some Blood Cells in Alloxan Induced Diabetic Rats

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Abstract

Impaired wound healing process is recognized as one of the major problems resulting severe complications of diabetes mellitus (DM). The work was designed to study the hyperglycemic activity and influence on some blood cells of leaf extracts of *Azadirachta indica* and *Mangifera indica* in diabetic rats. Adult healthy rats of both sexes about eleven weeks old were randomly assigned into six groups following acclamation to laboratory and handling conditions. Diabetes was induced with a single dose of alloxan (100mg/kg bw) and serum glucose was taken 72h after induction to confirm diabetes. Treatment regime of hyperglycemia and influence on platelet, wbc total and wbc differencial parameters started on the 4th and 9th day of the experiment respectively. The result obtained from the phytochemical analysis showed that the aqueous extracts of *Azadirachta indica* and *mangifera indica* gave positive reactions for alkaloids, tannins, flavonoids, saponins and phytosterols. The extracts decrease blood glucose in a dose dependent fashion.The results showed that wbc (total) and platelet count in the alloxan monohydrate intoxicated animals were significantly ($p<0.05$) increased in comparison to the normal animals. The treatment with vitamin C (reference drug) showed significant ($p<0.05$) decrease and increase in wbc (total) and platelet count respectively. In leaf extract of *Azadirachta indica* treatment, wbc (total) and platelet count decrease and increased respectively. *Mangifera indica* extract had more or less the same treatment response with vitamin C in decreasing and increasing wbc total and platelet count, thus, alleviating inflammation and oxidative damage.

1. Introduction

Diabetes mellitus (DM) is a complex disorder resulting from either insulin insufficiency or insulin resistance. It is characterized by disturbances of carbohydrate, protein and lipid metabolism [1]. Type 1 diabetes mellitus (Insulin dependent) is due to insulin deficiency because of lack of functional beta cells of the pancreatic gland. Patients suffering from type 1 DM are therefore totally dependent on exogenous source of insulin [2]. Type 2 diabetes (Insulin independent) develops as a result of insulin resistance. Patients of type 2 DM are unable to respond to insulin and can be treated with dietary changes, medication and exercise [2].

DM is a leading cause of death in the world [3]. In uncontrolled hyperglycemia conditions, varieties of metabolic complications are observed. Lipid abnormalities are common among other metabolic complications and may stand as the root cause of diabetes metabolic disorder. This diabetic metabolic disorder is apparent as lipoprotein lipase and cholesterylester hydrolase are very sensitive to insulin changes [1]. Therefore in insulin resistance and/or insulin deficiency extremely elevated triglyceride levels may result due to deactivated lipoprotein lipase. Similarly, increase levels of non-esterified fatty acids (NEFA) are released from the adipocytes due to activated of cholestrylase hydrolyase. This intervention will result in lowered levels of triglyceride clearance cultivating dyslipidemia.

Previous studies have shown that alloxan intoxication in albino rats produces significant dyslipidemia [1, 4]. Diabetes dislipidemia has been implicated in the progression of micro and macro vascular diseases leading to artherosclerosis and propagation of excessive inflammatory cascade, ultimately affecting angiogenesis [5-7]. Leucocytes recruitment to sites of inflammation is crucial for initiating immune mediated traffick [8]. This initiation cultivates adhesive tethering in the activated vascular wall [9]. Hence, insufficient angiogenesis plays a significant role in the pathogenesis of diabetic wound healing process [10].

Result of a number of studies have also shown that high glucose environment leads to formation of sugar derived substances called advanced glycation-end products (AGEs) that inhibit wound healing process [8, 11]. This

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eventually leads to generation of free radicals resulting in imbalance in the free radicals and antioxidants which induces oxidation stress and tissue damage followed by deficit in wound healing process [9].

Impaired wound healing process is recognized as one of the major problems resulting from severe complications of DM [12]. Wound may be defined as a loss or breaking of cellular and anatomic continuity of living tissue [13]. Whether or not the blood will coagulate after injury depend on the degree of balance between procoagulants and anticoagulants. Normally the anticoagulants predominate and the blood does not coagulate but when a vessel is reptured or is traumatized, procoagulants predominate in the area of damage and then a clot does develop [13]. Platelets are necessary for clot retraction to occur. Therefore failure of clot retraction is an indication that the number of platelet in the circulatory blood is low [14]. The healing process occurs in four stages including haemostasis (Platelet activation and blood clothing), inflammation (WBCs recruitment and phagocytosis), proliferation (Cell growth and tissue formation) and maturation (wound contraction and closure) [15]. It is well documented that the haemostasis and inflammation phases are altered or impaired in diabetic [16, 17]. This suggests that these processes may well take longer than healing would take in patients without diabetes.

Various traditional herbal-based supplements have been reported to exhibit curative value for various disorders [18]. For many years plants have been used as the remedy for various skin and dermatological disorders [19]. Considering the decreases quality of life and numerous comorbidities that are associated with diabetes, it is imperative that holistic and multi-factorial approach to treating diabetes and its complication be examined. Presently there is increasing evidence that many natural food supplements have the potential to become valuable complementary therapy in the treatment of DM and its complications.

Thus *Azadirachta Indica* (Neem) and *Mangifera indica* (Mango) are among the herbal remedies used for diabetes, rheumatic pain and fatigue in Nigeria.

Neem (*Azudirachta indica)* also known as Indian lilac is a tree in the mahogany family melilaceae while Mango (*Mangifera indica*) is a tree in the family anacardiaceae [20].

Thus this research is required to examine the antidiabetic activity of these herb extracts and their influence on some blood cells in alloxan-induced DM albino rats.

2. Materials and Methods

2.1. Materials and Drugs

Insulin (Novolog USA) and vitamin C (Emzor Nigeria) were used as test drugs. Alloxan monohydrates (St Louis MD USA) was used as induction drug. Accuchek active glucometer (Roche diagnostic) was used in glucose assay. All other reagents used in this study were of high analysis grade.

2.2. Plants Sample Collection

Fresh leaves of *Azadirachta indica* (Neem) and *Mangifera indica* (mango) were collected from tress located within the premises of Abia State Polytechnic Campus Aba Nigeria.

2.3. Preparation of Plants Aqueous Extracts

The healthy leaves of *Azadirachta indica* and *Mangifera indica* collected were properly washed under running water and air dried at room temperature for several days. The dried leaves were then pulverized using an electric slender and stored in an air tight container separately and kept in a cool-dry place for further analysis. A portion of each of the powered leaves (50g) was weighed and dissolved in 400ml of hot distilled water (40^0c-60^0c) and allowed to cool for 60 minutes and then filtered before being used in administration. These aqueous extracts were prepared daily to avoid turbidity and bacteria actions due to poor storage systems.

2.4. Preliminary Phytochemical Screening

Preliminary phytochemical screenings of *Azadirachta indica* and *Mangifera indica* were carried out to detect the presence of flavonoid, tannin, saponin and alkaloids and phytosterols using standard procedures as described by Ukpabi, *et al.* [21].

2.5. Experimental Protocol

2.5.1. Procurement of Animals

Adult healthy Albino rats (Wistar strain) of both sexes of about eleven weeks old with an average body weight of 120-200g were procured from Biochemistry Department University of Nigerian Nsukka. The animals were allowed to acclamatize for one week in the Department of Biochemistry of the Abia State Polytechnic, Aba Abia State. They were kept in different cages with high hygiene and of standard housing conditions of temperature (22 to 28^0C) and 12hours light/12 hour dark regime. They were fed with standard rat feed and water libitum throughout the duration of the study.

2.5.2. Induction of Diabetes Mellitus

The protocol for induction of diabetes has been observed and used with little modifications. The animals of all groups except normal control were weighed and fasted overnight before administration of alloxan. Diabetes was induced by single dose of alloxan (100mg/kg body weight) injected intrapertorially. The fasting blood sugar was measured on the 3rd day after alloxan injection by glucometer to ensure alloxan induction. Rats with blood glucose level >250mg/dl were considered as diabetic and induced in the study.

2.5.3. Experimental Design

Total of 48 rats were used and randomly divided into 6 groups each group containing six rats (table 1)

Table-1. Grouping of animals

Groups	Name	Treatment (n=6)
Group A	Normal control (NC)	Normal rats without intoxication giving food and water
Group B	Diabetic control (DC)	Diabetic rats giving food and water
Group C	Diabetic standard 1	Diabetic rats treated with insulin (40mg/kg)
Group D	Diabetic standard 2	Diabetic rats treated with vitamin C (10mg/kg)
Group E	Diabetic test 1	Diabetic rats treated with 200mg/kg extract of the herb (low dose)
Group F	Diabetic test 2	Diabetic rats treated with 400mg/kg extract of the herb (high dose)

2.5.4. Treatment

Diabetes was induced three days period to the treatment. The treatment regime of hyperglycemia started on the 4^{th} day and was given insulin (40mg/kg) and the respective herbs (200 and 400mg/kg). Blood glucose estimation were done on the 6^{th}, 9^{th} and 12^{th} day of the study.

Blood cells evaluation started on the 8^{th} day and was given vitamin C (10mg/kg) and the respective herbs (200 and 400mg/kg). WBC (total and differential) and platelet count were estimated on the 11^{th} and 17^{th} day of the study.

2.5.5. Biochemical and Hematological Parameters

At the end of the experiment, the rats were fasted overnight and samples of blood were obtained through cardiac puncture. Blood was collected into hepannized tubes. Serum was separated by centrifugation and used for estimation of glucose level according to Ukpabi, *et al.* [22]. Platelet, WBC total and differential were estimated according to procedures of Cheesbrough [23].

2.5.6. Statistical Analysis

Results from the analysis were expressed as Mean +SD and $p<0.05$ being considered as statistically significant.

3. Result

3.1. Phytochmical Screening of the Two Leaves Extracts

The result obtained from phytochemical screening showed that the aqueous extracts of *Azadirachta indica* and *Mangifera indica* gave positive reactions for alkaloids, tannins, flavonoids, saponins and physterols.

Table-2. Phytochemical Screening of the Two Leaves Extracts

	Phyto-constituents	Test	Results	
			Azadirachta indica	*Mangifera indica*
1.	Alkaloids	a) Mayers test b) Murexide test	Present Present	Present Present
2.	Tannins	a) 5% $FeCl_3$ b)Dilute $HN0_3$	Present Present	Present Present
3.	Flavonoids	a)Lead acetate b)Sodium hydroxide	Present Present	Present Present
4.	Saponins	a)Frothing b)Emulsion test	Present Present	Present Present
5.	Phyosterols	a)Solkowski test b)Libermann Burchards test	Present Present	Present Present

3.2. Effects of Aqueous Leaves Extracts of Azadirachta Indica and Mangifera Indica on Blood Glucose Concentration of Alloxan Induced Albino Rats

The result showed that leaves extracts of *Azadirachta indica* and *Mangifera indica* decreased the induced blood glucose levels in a dose dependent fashion. *Mangifera indica* leaf extract showed more hypoglycemic effect than *Azadirachta indica*.

Table-3. Effects of aqueous leaves extracts of *Azadirachta indica* and *Mangifera indica* on blood glucose concentration of alloxan induced albino rats

Group	Initial glucose Conc. (mg/dl)	Glucose conc. after 6days of treatment(mg/dl)	Glucose conc. after 9days of treatment(mg/d)	Glucose conc. after 12days of treatment(mg/dl)
A	108.25 ±3.27	102.40±3.20	106.48±1.98	110.51±2.90
B	313.75 ±1.92	291.58±4.32	302.23±2.59	298.85±3.42
C	313.75±1.31	196.58±1.92	118.25±1.33	99.25±1.91
E_1 LD	302.75±4.38	272.75±5.12	198.75±1.92	127.25±2.59
E_2 HD	302.75±4.38	216.50±2.06	170.00±2.24	109±4.97
F_1 LD	306.25±6.50	217.75±2.49	147.00±4.06	101.75±4.92
F_2 HD	302.25±6.50	151.50±2.96	95.25±4.09	70.50±2.60

Result are expressed as mean ±SD

3.3. The Effect of Alloxan Monohydrate Induction on Platelets Count, White Blood Cell, Total and Differential of the Albino Rat

The result showed that platelet count, wbc total and differential in the alloxan monohydrate intoxicated animals were significantly ($p<0.05$) increased in comparison to the normal animals.

Table-4. The effect of alloxan monohydrate induction on platelets count, white blood cell, total and differential of the albino rats

	(Cells/l) w.b.c	(Cells/ml) Platelets
Group A	2550.0±0.00	242.5±7.50
Group B	11250.0±0.00	251.5±1.20

Result are expressed as mean ±SD

3.4. The Effect of Vitamin C and Aqueous Leaves Extracts on Platelet Count, White Blood Cell, Total and Differential of Diabetic Albino Rats

Treatment with vitamin C (reference drug) and *Mangifera indica* showed significant ($p<0.05$) decrease and increase in white blood cells (total) and platelet count respectively during the period of treatment. In leaf extracts of *Azadirachta indica* treatment, white blood cells (total) and platelet count showed significant increase.

Table-5. The effect of vitamin C and aqueous leaves extracts of the two herbs on platelet count, white blood cell, total and differential of diabetic albino rats

	w.b.c total (cells/l)		w.b.c differential				Platelet(cells/ml)	
	11^{th} day	17^{th} day	11^{th} day		17^{th} day		11^{th} day	17^{th} day
			N	L	N	L		
Vitamin C	6.810±3.21	4375±2.00	13%	87%	5%	95%	277.5±1.21	342.5±2.00
E1	19975±5.50	25070±7.84	4%	96%	6%	94%	325±20.6	336±5.08
E2	18375±6.66	21125±7.94	12%	88%	8%	92%	430±4.38	550±4.92
F1	9800±20.05	3850±20.00	8%	92%	12%	88%	285±3.20	315±2.50
F2	10600±20.00	5650±15.50	9%	91%	8%	92%	292±2.00	321±2.40

Result are expressed as mean ±SD

4. Discussion

Diabetes mellitus is a metabolic disorder characterised by hyperglycemia which predisposes sufferers to chronic complications affecting several organs of the body, including the eye, blood vessels, kidney and the nerves [24]. It can also have significant impact on wound healing process Blakytny and Jude [25]. Identifying and taking action on risk factors recognized in diabetic patients may reduce the number of wounds that develop in diabetic patients and also reduce the time it takes for these wounds to heal. Vascular and micro circulatory changes among others may play part in both the causation and impaired healing of wounds in the patients with diabetes [8]. Understanding the alteration in blood cells in the diabetic albino rats is of great importance to help identify practical solutions which may reduce the incidence and wound healing process. The influence of leaves extracts of *Azadirachta indica* and *mangifera indica* on blood glucose level and some blood cells in alloxan induced diabetic rats was investigated.

Plant products have been universally accepted as potential wound healing agents for years due to their profound availability of bioactive compounds with minimal toxi city, and their usage at crude preparation [26].

The two herbs, *Azadirachta indica* and *Mangifera indica* on phytochemical screening showed the presence of flavonoids, tannins, saponins, alkaloids and phytosterols as shown in table 2. Previous experimental investigations have shown that these various secondary metabolites promote wound healing through different mechanisms Ali and Ibiam [27]. These secondary metabolites observed in this study have been shown to posses anti-oxidants, anti-microbial and free radical scavenging activities [28]. Other studies also revealed their abilities to reduce lipid peroxidation, show anti-inflammatory and adaptogenic activities [14]. Plants especially these *Azadirachta indica* and *Mangifera indica* provide good environment for tissue healing via the presence of these bioactive metabolites. These bioactive metabolites generally promote wound healing by reducing lipid per-oxidation, thereby improving vascular and preventing or slowing down the process of cell necrosis [29]. Thus increased levels of these bioactive

metabolites lead to increase in the viability and strength of collagen fibrils along with improved blood circulation [30].

The effect of alloxan monohydrate (100mg/kg) intoxication on blood glucose, leukocytes, and platelet cells in the albino rats showed significantly increase in this study.

Diabetes increase the risk of plaque buildup in the arteries which can cause dangerous blood clots. When vascular changes occurs, inflammation follows [31]. Inflammation is characterized by clotting of the fluid in the intestinal spaces and migration of large numbers of leucocytes into the tissue.

The result also showed on treatment, that the fasting blood glucose levels were significantly reduced in oral administration of the two leaves extracts of *Azadirachta indica* and *mangifera indica* in a dose dependent manner. This property may be beneficial in different stages of wound healing process by exhibiting hypoglycemic activity. Since control over blood glucose levels have been shown to improve wound healing in diabetes [11].

The table 3 presented the effect of alloxan monohydrate intoxication in leucocytes and platelets cells. There were significantly increase in the concentrations of the leucocytes and platelets cells compared to the control. These cells have been implicated in the wound healing process. The two major leucocytes induced in the alloxination response are lymphocytes and neutrophils.

Mangifera indica showed significant increase in the platelet count and significant decrease in white blood cells with a relative rise and fall in the lymphocytes and neurophils. *Azadirachta indica* showed increase in both platelet count and white blood cells.

Platelets may assume an important role in atherosclerosis in diabetes. Vascular change play role in the increased clotting factors and activation of platelets seen in diabetes [32]. Although blood clots routinely form as a normal function of blood cells to repair damaged blood vessel walls, clots become a problem when they prevent blood from flowing through an artery or vein inappropriately. After inflammation, large numbers of lymphocytes and neurophils begin to invade the area. This is caused by products for the inflammed tissue. These cells strongly activate the macrophage system and devour the destroyed tissue.

The reference drug (vitamin C) showed increase in platelet count and decrease in the white blood cells with relative rise in lymphocytes. Sharma, *et al.* [14], made a similar observation on treating alloxan rats with vitamin C extracts. The clinical manifestation of scury hemorrhage from membrane of the mouth and gastrointestineal tract, anaemia, pains in the joints can be related to the association of ascobic acid and normal connective tissue metabolism [33]. This function of ascorbic acid also accounts for the requirement for normal healing.

This study showed similar treatment response between *Mangifera indica* and vitamin C. Vitamin C has been shown to have anti oxidative and anti-inflammatory function [14]. It plays a great role in the synthesis of collagen by maintaining the necessary enzymes in their active form. The vitamin C extracts may help to prevent inflammatory and oxidative damage which alternatively promote wound healing process. Decrease in wbc with increase in platelet count may help ameliorate the inflammation of mucus membrane of the vascular system and prevention of oxidative damage. Alternatively an elevation in white blood cells (total) in *Azadirachta indica* treatment group may be as a result of immunity boosting property.

5. Conclusion

Mangifera indica leaf extract and vitamin C, may have prevented inflammation and oxidative damage as documented by significant reduction in WBC (total) and increase in platelet count in diabetic rats. Alternatively an elevation in white blood cells (total) and platelet cells in *Azadirachta indica* treatment group may be as a result of immunity boosting property. These environment may ultimately promote wound healing process.

References

[1] Ukpabi, Nwankwo, J., Eshie, T., and Isu, J., 2018. "Extract of vernonia amygdalina on blood glucose and lipid profile of alloxan-induced diabetic rats." *Iosr Journal of Pharmacy and Biological Sciences*, vol. 13, pp. 13-17.

[2] Nagashayana, G., Jagadeesh, K., and Shreenivas, P. R., 2014. "Evaluation of hyperglycemic activity of neem (azadiratchta indica) in Albino rats." *IOSR Journal of Dental Sciences*, vol. 13, pp. 04-11.

[3] Jisieke-Onuigbo, N. N., Unuigbe, E. I., and Oguejiefor, 2011. "Dyslipidemias in type 2 diabetes milletus in nnewi South east Nigeria." *Annals of African Medicine*, vol. 10, pp. 285-289.

[4] Ukpabi, Chukwu, M. N., Onyemachi, J. N., Ibe, P., and Onuh, E. F., 2019. "Antidiabetic and anti hyperlipidemic effects of aqueous extract of carica papaya leaf on the experimental model against single alloxan toxicity." *World Scientific Research*, vol. 6, pp. 14-18.

[5] Altabas, V., 2015. "Diabetes, endothelial dysfunction, and vascular repair. What should a dialectologist keep his eye on?" *Int. J. Endocrinol.*, pp. 1-14. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4452196/

[6] Okonkwo, O. U., Chen, L., Modilevsky, B., Zhao, Y., and Dipietro, L. A., 2017. "Vascular maturity and integrity in Diabetic skin wounds." In *wound Repair and Regeneration; Davidson J. M. Ed, wound healing society: San Diego, C.A USA, pp W7-W8.*

[7] Dipietro, L. A., 2016. "Angiogenesis and wound repair: When enough is enough." *J. Leukoe. Biol.*, vol. 100, pp. 1-6.

[8] Tam, J. C. W., Lau, K. M., Liu, C. L., To, M. H., Kwoh, H. F., Lai, K. K., Lau, C. P., Ko, C. H., Leung, P. C., *et al.*, 2011. "The in vivo and in vitro diabetic wound healing effects of a 2-herb formula and its mechanisms of action." *J. Ethnopharmacol.*, vol. 134, pp. 831-8.

[9] Murthy, S., Gautam, M. K., Goel, P. V., Sharma, H., and Goel, R. K., 2013. "Evaluation of in vivo wound healing activity of Bacopa monniera on different wound model in rats." *Biomed Res Int.,* pp. 1-9. Available: https://www.ncbi.nlm.nih.gov/pubmed/23984424

[10] Dimmeler, S. and Zeiher, A. M., 2000. "Endothelial cell apoptosis in angiogenesis and vessel regression." *Circ. Res.,* vol. 87, pp. 434-439.

[11] Tuhin, R. H., Begum, M., Rahman, S., Karim, R., Begum, T., Ahmed, S. V., Mosfofa, R., Hossain, A., Abdel, D., M., *et al.*, 2017. "Wound healing effect of Euphobia hirta Linn (Euphorbiaeceae) in alloxan induced diabetic rats." *BMC Complement Altern Med.,* vol. 24, p. 423.

[12] Falanga, V., 2005. "Wound healing and its impairment in the diabetic foot." *Lancet.,* vol. 366, pp. 1736-1743.

[13] Kokane, D. D., More, R. Y., Kale, M. B., Nehete, M. N., Mehendale, P. C., and Gadgoli, C. H., 2009. "Evaluation of wound healing activity of root of Mimosa pudica." *J. Ethnopharmacol.,* vol. 124, pp. 311-5.

[14] Sharma, N., Samarakoon, K. W., Gyawali, R., Park, Y. H., Lee, S. J., Oh, S. J., Lee, T. H., and Jeong, D. K., 2014. "Evaluation of the antioxidant, anti-inflammatory, and anticancer activities of Euphorbia hirta ethanolic extract." *Molecules,* vol. 19, pp. 1456-81.

[15] Nayak, B. K., Sandiford, S., and Maxwell, A., 2009. "Evaluation of the wound healing activity of ethanolic extract of morinda citrifolia leaf evid-based complement altern." *Med,* vol. 6, pp. 351-6.

[16] Lioupis, C., 2005. "Effects of diabetes mellitus on wound healing: an update." *Journal of Wound Care,* vol. 14, pp. 84-86.

[17] Kidman, K., 2008. "Tissue repair and regeneration the effects of diabetes on wound healing diabetic foot." *Journal,* vol. 11, pp. 73-79.

[18] Galehdari, H., Negahdari, S., Kesmati, M., Rezaie, A., and Shariati, G., 2016. "Effect of the herbal mixture composed of aloe vera, henna, Adiantum Capillus-veneri, and Myrrha on wound healing in Streptozotocin-induced diabetic rats." *BMC Complement Altern Med,* vol. 16, p. 386.

[19] Kumar, B., Vijayakumar, M., Govindarajan, R., and Pushpangadan, P., 2002. "Ethnopharmacological approaches to wound healing-exploring medicinal plants of India." *J. Ethnopharmacol.,* vol. 114, pp. 103-13.

[20] Folkman, J., 1995. "Angiogenesis in cancer, vascular, rheumatoid and other disease." *Nat. Med.,* vol. 1, pp. 27-31.

[21] Ukpabi, Agbafor, K. N., Ndukwe, O. K., Agwu, A., and Nwachukwu, S. N., 2012. "Phytochemical of coustus after extract and its alleviation of carbon tetrachloride -induced hepatic oxidative stress and toxicity." *International Journal of Modern Botany,* vol. 2, pp. 120-126.

[22] Ukpabi, Chibueze, Stephen, Chinwendu, Onyeji, Maryjane, and Ezeigbo, R. O., 2015. "Effects of Biherbal Aqueous extract of Vernonia amygalina (Bitter leaf) and Gongronemalatifolium (utazi leaf) on alloxan induced diabetic rats." *International Journal of Scientific Research and Engineering Studies,* vol. 2, pp. 136-140.

[23] Cheesbrough, M., 2000. *Medical laboratory for tropical countries. Microbiology*: Linarce House, Jordan Hill oxford. pp. 260-287.

[24] Ahmed, 2005. "Advanced glycation endproducts-role in pathology of diabetic complications." *Diabetic Research and Clinical Practice,* vol. 67, pp. 3-21.

[25] Blakytny, R. and Jude, E., 2006. "The molecular biology of chronic wounds and delayed healing in diabetes." *Med,* vol. 23, pp. 594-608.

[26] Micera, A., Vigneti, E., Pickholtz, D., Reich, R., Pappo, O., and Bonini, S., 2001. "Nerve growth factor displays stimulatory effects on human skin and lung fibroblast demonstrating a direct role for this factor in tissue repair." *Proc. Natl. Acad. Sci.,* vol. 98, pp. 6162-7.

[27] Ali, F. U. and Ibiam, U. A., 2014. "Phytochemical studies and gc-ms analysis of gongroneme latifolium and piper civic neense." *International Journal of Innovate Research and Development,* vol. 3, pp. 108-114.

[28] Mikhal'chik, E. V., Anurov, M. V., and Titkova, S. M., 2006. "Activity of antioxidant enzymes in the skin during surgical wounds." *Bull exp Biol Med.,* vol. 142, pp. 667-9.

[29] Schmidt, C., Fronza, M., and Goeltert, M., 2009. "Biological studies on Brazilian plants used in wound healing." *J Ethnopharmacol,* vol. 122, pp. 523-32.

[30] George, B. P., Parimelazhagan, T., Kumar, Y. T., and Sajeesh, T., 2003. "Antitumor and wound healing properties of rubus ellipticus smith." *J. Acupunct Meridian Study,* vol. 8, pp. 134-41.

[31] Martin, P. and Leiborish, S. J., 2005. "Inflammatory cells during wound repair: The good, the bad and the ugly." *Trends Cell Boil,* vol. 15, pp. 599-607.

[32] Gwonda, A. C., Kumar, N. B., Kasture, P. N., and Nagabhushan, K. H., 2015. "A pilot study to evaluate the effectiveness of Carica papaya leaf extract in increasing the platelet count in cases of dengue with thrombocytopenia." *Indian Med Gaz. CXLIX,* pp. 109-16.

[33] Yechoor, V. K., Patti, M. E., Saccone, R., and Kahn, C. R., 2002. "Coordinated patterns of gene expression for substrate and energy metabolism in skeletal muscle of diabetic mice." *Proc. Natl. Acad. Sci. USA.,* vol. 99, pp. 10587-10592.