World Journal of Nutrition and Health, 2015, Vol. 3, No. 3, 47-52
Available online at http://pubs.sciepub.com/jnh/3/3/1

DOI:10.12691/jnh-3-3-1

Glycemic and Wound Healing Effects of Aqueous Mesocarp Extract of Unripe *Carica papaya* (Linn) in Diabetic and Healthy Rats

Magnus Michael Chukwudike Anyakudo*, Adeife Benjamin Erinfolami

Endocrinology/Metabolism and Nutrition Research Unit of Department of Physiology, Faculty of Basic Sciences, College of Health Sciences, Bowen University Iwo, Osun State, Nigeria
*Corresponding author: micmagkudos@yahoo.com

Received August 24, 2015; Revised September 07, 2015; Accepted September 29, 2015

Abstract This experimentally-controlled designed study investigated the wound healing and glycemic effects of aqueous mesocarp extract (AME) of unripe *Carica papaya* (CP) in alloxan-induced diabetic and healthy rats with the rationale of providing cost effective and accessible alternative wound phytotherapy and glycemic control. Five groups (n=6, each) of adult male Wistar albino rats were used in this study which lasted for 21 days: DC group treated with olive oil, DT group treated with AME, NC group treated with olive oil, NT group treated with AME and PC group treated with tincture of iodine. A full thickness excision wound of circular area 300 mm^2 and 2mm in-depth was inflicted on all rats after light anesthesia with i.v. ketamine (120mg/kg b.w.) Diabetes was induced with alloxan monohydrate (150mg/kg, i.p). The extract was topically applied to the opened wounds at dose of 100 mg $kg^{-1}day^{-1}$ for 3 weeks. Wound areas were measured on days 1, 7, 14 and 21 using a transparent sheet and a permanent marker. Assessment of wound healing activity was made using percentage area of wound contraction, epithelization period and granulation tissue integrity. Glycemic impact of orally administered dose of 200 and 400mg/kg/day extract was assessed in all experimental groups using glucometer. Results were expressed in mean±SEM and comparison between groups was made using one way ANOVA. P values of < 0.05 were considered statistically significant. Significant ($p < 0.05$) increase in percentage area of wound contraction was observed in DT (97%) and NT (97%) rats with well organized granulation tissues and remarkable earlier epithelization (day 14) compared with their respective control (DC - 77%; NC - 92%) with delayed epithelization (day 17-21). A significant dose-dependent hypoglycemia was observed in extract treated rats. Unripe *carica papaya* L. aqueous mesocarp extract induced remarkable hypoglycemic effect with effective wound healing potential in alloxan-induced diabetic and healthy rats.

Keywords: *aqueous mesocarp extract, Carica papaya Linn, diabetic rats, glycemic control, wound healing*

Cite This Article: Magnus Michael Chukwudike Anyakudo, and Adeife Benjamin Erinfolami, "Glycemic and Wound Healing Effects of Aqueous Mesocarp Extract of Unripe *Carica papaya* (Linn) in Diabetic and Healthy Rats." *World Journal of Nutrition and Health*, vol. 3, no. 3 (2015): 47-52. doi: 10.12691/jnh-3-3-1.

1. Introduction

Delayed wound healing and chronic wound management in diabetics is of public interest due to their social and economic burden on the individuals and care givers [1]. Diabetic wounds are chronically slow healing wounds that are difficult to manage despite adequate and appropriate care [2]. Though, the exact pathogenesis of poor and delayed wound healing in diabetics is not clearly understood, however, evidences from human and animal studies have revealed several abnormalities in the various phases of the wound healing process implicated by uncontrolled hyperglycemia [3,4,5]. As a result, affected individuals spent large sum of money on hospital bill to procure adequate management coupled with long standing hospital admission. To abate such demands, a paradigm shift to alternative medicine is recently attracting focus. Different parts (fruit, leaf, stem, latex, root) of a tropical tree called *Carica papaya* Linn (family: Caricacaea) with over 1000 species worldwide [6] have long been reported to be used as herbal medicines or concoctions in the management of various disorders including diabetes and wound infection [7]. Use of *Carica papaya* various parts, which are variably rich in Vitamin A and C and other several biochemical compounds [8,9], have been reported useful in the treatment of various disorders [10] in healthy and diabetic subjects. However, the use of its mesocarp (ripe or unripe) in wound healing and glycemic control in diabetics has not been adequately explored as evidenced by dearth of available reports. This experimentally-controlled designed study therefore investigated the glycemic and wound healing effects of aqueous extract of unripe *Carica papaya* mesocarp in male Wistar diabetic rats with the rationale of providing cost effective and accessible alternative wound phytotherapy and glycemic control in diabetics.

2. Materials and Methods

2.1. Plant Materials

The fruits of CP were collected from the campus of Bowen University College of Health Sciences Nigeria after the authentication of the plant's identity by a botanist. Voucher specimen was deposited in the institution herbarium.

2.2. Preparation of the Extract

The unripe fruits of the plant was washed and properly cleaned with distilled water. The outer green thin layer (exocarp) was peeled and discarded. The underlying mesocarp was peeled and weighed. 200g of the peeled mesocarp was blended with 50 ml of distilled water to a fine texture form using a laboratory blender. The mixture obtained was filtered using a fine muslin cloth to produce a residue that was oven dried at 40°C to a white colored powdery form. The powdered residue was weighed and stored in air and water proof containers, kept in refrigerator at 4°C. Portion used for oral administration to assess glycemic impact was prepared fresh from this stock whenever required.

2.3. Animals

Healthy male Wistar (*Rattus norvegicus*) albino rats weighing about 200 - 250 g were purchased from the disease-free stock of the animal house of the department of Biochemistry, Bowen University, Iwo, Osun state, Nigeria and were used for the study. The animals were housed in poly propylene cages, maintained under standard conditions (12:12 h light:dark cycle; 25 ± 2°C, relative humidity). They were fed with standard rat pellet diet (Oladokun Feed Ltd. Ibadan, Nigeria) and water *ad libitum.* The Institutional Animal Ethical Committee of Bowen University Iwo, Nigeria approved the study protocol.

2.4. Sample Collection

Blood samples were collected by tail snipping method from the cordal veins of the overnight fasted (15 hrs) rats and blood glucose levels were estimated using On Call Plus® glucose strips and test meter device (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA), which measures the blood glucose level by glucose oxidase-peroxidase method.

2.5. Toxicity Test

An acute toxicity test using stair-case method [11] was conducted for the extract. The animals were provided orally with increasing doses of 1, 2 and 3g/kg body weight of the extract while the toxicity was assessed by mortality and behavioral changes of the rats.

2.6. Induction of Diabetes

A single dose (150 mg/kg, b.w., i.p.) of alloxan monohydrate (Sigma Ltd., USA) dissolved in normal saline was used for induction of type 2 diabetes in rats after overnight fasting. After 1 h of alloxan administration, the animals were fed standard pellets and water *ad libitum.* The animals were stabilized for a week and animals showing blood glucose level more than 250 mg/dL were selected for the study.

2.7. Experimental Design

Rats fasted overnight for 15hrs were randomly divided into 5 groups of 6 rats each. NC group served as non-diabetic (normal) control which received topical olive oil. NT group served as normal treated which received topical extract. PC group served as non-diabetic positive control treated with tincture of iodine. DC group served as diabetic control which received topical olive oil and DT group served as diabetic treated which received topical extract. NT, DT and PC Groups were treated orally with 200, 200, and 400 mg/kg/day of aqueous mesocarp extract of *Carica papaya* Linn (AMECPL) respectively based on their acute oral toxicity study while NC and DC groups served as control receiving 10mL/kg b.w of distilled water. Blood glucose estimation was done at 0 (fasting), and subsequently hourly for 4 hours post treatment. Treatment was continued for 21 consecutive days. Fasting blood glucose levels were estimated weekly for 3 weeks i.e. at 1 (entry), 7, 14, and 21 days.

2.8. Wound Infliction and Treatment

Rats were inflicted with excision wounds according to Morton and Malone [12] method. Animals were anaesthetized with 0.48 ml of intravenous ketamine hydrochloride (120 mg/kg body weight) and shaved on the back using scissors. The area of the wound to be created was outlined on the back of the animals with a blue ink marker. A full thickness excision wound of circular area 300 mm^2 and 2mm in depth was created along the markings. The entire wound was left open. None of the animals showed any signs of infection as they were closely observed. The animals were divided into groups according to experimental design above. The treatment was done topically in all experimental groups. The extract was applied at dose of 100 mg $kg^{-1}day^{-1}$ for 21 days. Wound areas were measured and recorded on days 1, 7, 14 and 21 for all the groups using a transparency sheet and a permanent marker.

2.9. Statistical Analysis

Data was analyzed using appropriate statistical methods and program of Microsoft Excel and SPSS v. 21. All the values were expressed as mean±SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Duncan's multiple range tests. $P < 0.05$ was considered significant.

3. Results

3.1. Toxicity Evaluation

Administrations of single dose of extract (200-400 mg/kg, b.w., and p.o.) did not produce any mortality. All the 6 animals were alive, healthy and active during the observation period of 21 days. Acute toxicity studies revealed that extract doses of up to 3g/kg b.w. *per oris* was the peak dose that produced no signs of toxicity and mortality. All the animals were found to be alive, healthy and active during the observation period of 21 day postadministration of highest dose.

3.2. Wound Healing Effect of AMECPL in Experimental Rats

The wound healing activity of the AMECPL is shown in Table 1 and Table 2 and Figure 1, Figure 2, Figure 3 and Figure 4.

Table 1. Wound healing activity of AMECPL (100mg/kg/day) in grouped experimental rats (n = 6/group)

Periods	Wound Contraction Area (mm^2) in Grouped Experimental rats				
	NC/olive	NT/Extract	PC/iodine	DT/Extract	DC/Olive
Day 1	346.5±0.65	346.5±0.65	346.5±0.65	346.5±0.65	346.5±0.65
Day 7	176.8±0.26	154.0±0.19	95.07±0.23	113.1±0.20	254.6±0.31
Day 14	28.3±0.07	7.07±0.01	3.14±0.16	7.07±0.01	78.57±0.18
Day 21	18.2± 0.05	3.14±0.02	1.12±0.21	3.64±0.23	40.24±0.16

Values are expressed in mean ± SEM.

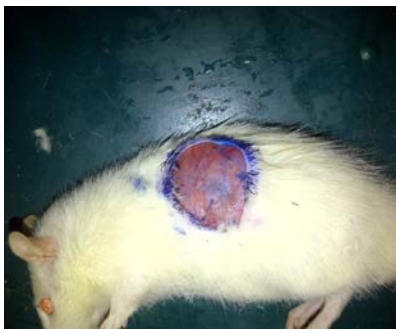

Figure 1. Anaesthetized rat with inflicted wound on day 1

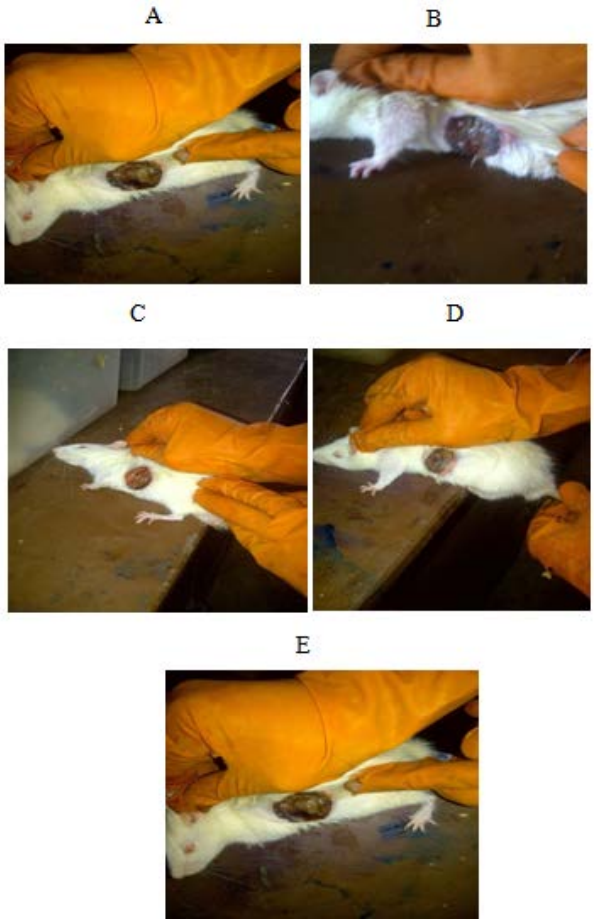

Figure 2. Grouped experimental rats showing areas of wound contraction on day 7

A-Diabetic rats treated with extract.
B-Diabetic rats treated with olive oil.
C-Normal rats treated with extract.
D-Normal rats treated with iodine (NCP).
E Normal rats treated with olive oil.

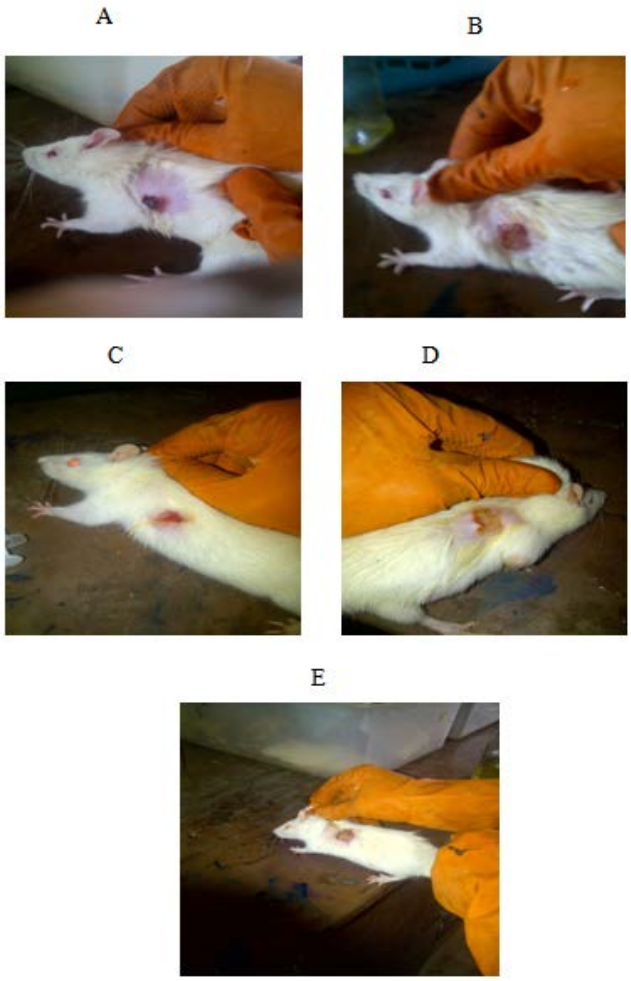

Figure 3. Grouped experimental rats showing areas of wound contraction on day 14

A-Diabetic rats treated with extract.
B-Diabetic rats treated with olive oil.
C-Normal rats treated with extract.
D-Normal rats treated with iodine (NCP).
E Normal rats treated with olive oil.

3.2.1. Wound Contraction Area (mm^2) and Epithelization

The healing activity of AMECPL on the wound of diabetic rats is shown in Table 1. Rats treated with AME showed significant ($P < 0.05$) increase in percentage area of wound contraction (DT - 97%) and NT- 97%) with well organized granulation tissues and remarkable epithelization on day 14 compared with their respective controls treated with olive oil (DC - 77%; NC – 92%) with remarkable epithelization on day 17-21 (Table 2). Healing rate was

however faster in healthy rats compared with the diabetic rats. Differences in wound healing activity between AME treated rats and positive (standard) control rats were insignificantly comparable.

Table 2. Period of remarkable epithelization in experimental rats (n = 6/group)

	Experimental groups				
	NC	**NT**	**PC**	**DT**	**DC**
Period of epithelization (Days)	17.50±.02	15.1±.01	14.0±.00	15.2±.016	21.20±.015

Values are expressed in mean ± SEM.

3.3. Glycemic Effect of AMECPL in Experimental Rats

The hypoglycemic activity of AMECPL on the blood sugar level of healthy and diabetic rats is shown in Table 3 and Figure 5. Acute and chronic treatment with AMECPL in the doses of 200 and 400mg/kg b.w., in diabetic and healthy rats showed a significant ($P < 0.05$) decrease in the elevated blood glucose level as compared with their control. The extract in the dose of 200 mg/kg b.w. showed significant ($P < 0.05$) hypoglycemic activity from day 14 while that of 400 mg/kg b.w., showed significant ($P<0.05$) result from day 7.

Table 3 Effect of AMECPL on fasting blood (venous) glucose concentration in experimental rats (n = 6/group)

	Blood glucose concentrations (mg/dL)			
Experimental groups	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Normal control	88.9±1.3^{a}	89.2±1.2^{a}	88.5±1.6^{a}	90.14±1.4^{a}
Diabetic control	260.8±6.2^{a}	265.2±6.6^{b}	267.4±7.2^{b}	270.3±7.7^{c}
Normal + extract (200mg/dL)	89.2±1.6^{a}	83.4±1.2^{a}	75.1±0.4^{b}	72.2±0.8^{b}
Normal + extract (400mg/dL)	88.6±1.2^{a}	76.4±1.4^{b}	70.6±1.6^{c}	60.4±0.8^{d}
Diabetic + extract (200mg/dL)	258.2±5.2^{a}	250.4±5.0^{c}	240.2±4.4^{d}	216.2±2.2^{e}

Values are expressed in mean ± SEM. P values < 0.05 are significant. Values with different superscripts in a row or column are significant when diabetic control group is compared with diabetic + extract (200mg/dL) group; normal + extract (200mg/dL and 400mg/dL) groups compared with normal control and normal + extract (200mg/dL) compared with normal + extract (400mg/dL).

Figure 4. Grouped experimental rats showing areas of wound contraction on day 21

A-Diabetic rats treated with extract.
B-Normal rats treated with extract
C- Diabetic rats treated with olive oil.
D-Normal rats treated with iodine (NCP).
E Normal rats treated with olive oil.

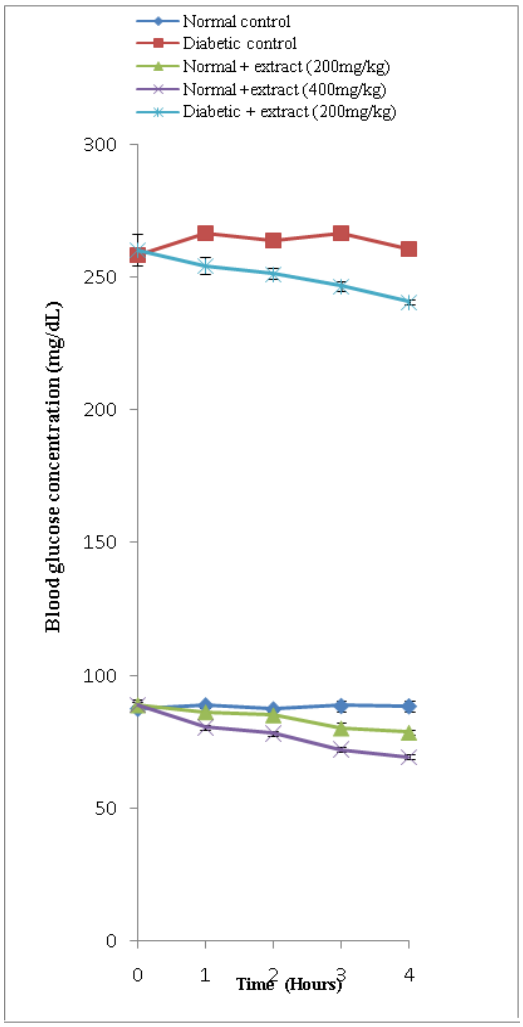

Figure 5. Glycemic profiles of experimental rats showing effects of AMECPL on blood glucose level (n = 6/group)

4. Discussion

This study revealed the phytotherapeutic and hypoglycemic potentials of aqueous mesocarp extract of *Carica papaya* Linn in wound healing and glycemic control both in healthy and alloxan-induced diabetic rats. The healthy and diabetic rats treated with AMECPL showed remarkable wound healing activity as expressed by the significant percentage reduction in wound contraction area (Table 1), remarkable epithelization (Table 2) and well-formed granulation tissue (Figures 3 and 4). However, rate of wound healing was faster in healthy rats than the diabetic counterpart. The dose-dependent hypoglycemic activity of the extract was remarkably expressed in this study when the measured values of the blood glucose concentrations following oral administration dose of 200mg/kg b.w. of the extract were compared with 400mg/kg b.w. in the healthy rats (Table 3). Meanwhile, administration of 200mg/kg b.w. of the extract in diabetic rats showed significant reduction in the blood glucose concentration from day 7 onward in a similar manner to the healthy rats' glycemic response.

Wound healing process is characterised by three stages such as inflammatory, proliferative and remodelling [13]. In the inflammatory phase, bacteria and debris are phagocytosed and removed, resulting in the release of factors that cause the migration and division of cells involved in the proliferative phase characterised by neoangiogenesis from endothelial cells. Diabetic wound healing has been reported generally slow if inappropriately cared for and this might last for weeks or even months with possibility of risk of amputation [14]. This above was observed in this study when the rate of wound healing activity was compared between the diabetic and healthy rats following topical application of the extract for 21 day study period. The healthy rats wound showed well-formed granulation tissue with remarkable epithelization and significant reduction in wound contraction area earlier (day 5-14) than the diabetic rats (day 14-21). This delayed wound healing may result from the metabolic effect of diabetes though; evidences from studies involving both human and animal models revealed possibilities of several abnormalities in the various phases of wound healing process [15,16].

The wound healing activity of this studied extract may be attributed to 'Papain', the active component of *C.papaya* L. that has been reported to provide enzymatic debridement of wounds in addition to the rich vitamin C content of the fruit essential for the conversion of proline to hydroxyproline a specific marker and a component of extracellular granulation tissue matrix in the wounds, indicating rapid turnover and accumulation that explains the increased rate of wound contraction [17]. In this study, remarkable granulation tissue proliferation was observed in diabetic and healthy rats treated with the extract. Compared with the positive control, the extract displayed insignificant difference in wound healing activity thus, reflecting the potentiality of the extract in wound healing.

In this study, aqueous mesocarp extract of *C. Papaya* induced significant reduction in blood glucose concentrations in healthy and diabetic (especially) rats (Figure 5). The observed hypoglycemic potential of the extract may be attributed to the enhanced secretion of insulin from the beta cells of the pancreas or increased tissue uptake of glucose by enhancement of insulin sensitivity. Though, the mechanism of the hypoglycaemic activity of the extract was not established in this study due to certain logistic reasons, however, further research on the organ effects of this extract is being necessitated to elucidate the mechanism at tissue level. While reports of the phytochemical analysis of *Carica papaya* fruits have revealed the presence of alkaloids, tannins, saponins, flavonoids, anthraquinones, anthocyanosides and reducing sugars, presence of any of these phytochemicals might be responsible for the antihyperglycemic activity of the extract in diabetic rats as reported by the finding of other study [18].

5. Conclusion

The results of this study demonstrated that C. papaya mesocarp extract when applied topically to wounds promotes wound healing remarkably in healthy and diabetic rats while its oral administration at nonlethal dose reduced blood sugar level effectively in diabetic rats.

Competing Interest

Authors have declared that no competing interest exists.

Acknowledgement

This work was carried out in collaboration between the authors. Author MMCA designed, supervised, performed the analysis and wrote the manuscript of the study while Author BEA assisted in the provision of essential materials and collection of samples data. Both authors read and approved the final manuscript.

References

[1] Williams, J.Z. and Barbul, A, "Nutrition and Wound Healing," *Journal Surg. Clin. North Am*., 83 (3). 571-96. June 2003.

[2] Falanga, V, "The chronic wound: impaired wound healing and solutions in the context of wound bed preparation," *Blood cells mol. dis.*, 32 (88). 2004.

[3] Goodson, W.H. and Hunt, T. K, "Studies of wound healing in experimental diabetes mellitus," *J. surg. res*, 22 (3). 221- 227. Mar. 1977.

[4] Bitar, M.S. and Labbad, Z.N, "Transforming growth factor-beta and insulin-like growth factor-I in relation to diabetes-induced impairment of wound healing," *J. Surg. Res*., 61(1). 113-119. Feb. 1996.

[5] Ekmektzoglou, A.K. and Zografos, G, "A concomitant review of the effects of diabetes mellitus and hypothyroidism in wound healing," *World J. Gastroenterol*., 12 (17). 2721-2729. May 2006.

[6] Marles, R.J. and Farnsworth, N.R, "Antidiabetic plants and their active constituents," *Phytomedicine*, 2 (2). 137-89. Oct. 1995.

[7] Maniyar, Y. and Bhixavatimath P, "Antihyperglycemic and hypolipidemic activities of aqueous extract of Carica papaya Linn. leaves in alloxan-induced diabetic rats," *J. Ayurveda Integr. Med*., 3(2). 70-74. Jun. 2012.

[8] Thomás, G.E., Rodolfo, H.G., Juan, M.D., Georgina, S.F., Luis, C.G., Ingrid, R.B, et al., "Proteolytic activity in enzymatic extracts from Carica papaya L. cv. Maradol harvest by-products," *Process Biochem*., 44. 77-82. 2009.

[9] Rivera-Pastrana, D.M., Yahia, E.M, and González-Aguilar, G.A, "Phenolic and carotenoid profiles of papaya fruit (Carica papaya L.) and their contents under low temperature storage," *J. Sci. Food Agric*., 90(14). 2358-65. Nov. 2010

[10] Gill, L.S, *Ethnomedical uses of plants in Nigeria. Benin, Nigeria*, Uniben. Press. 1992.

[11] Jalapure, S.S., Patil, M. B., Prakash, N. S., Hemalatha, K., Manvi, F. V, "Hepatoprotective activity of the fruits of *Piper longum* Linn.," *Indian J. Pharm. Sci.*, 65 (4). 363-366. 2003.

[12] Morton, J. J. and Malone, M.H, "Evaluation of vulnerary activity by an open wound procedure in rats," *Arch. Int. Pharmacodyn. Ther*., 196 (1) 117-126. Mar.1972.

[13] Stadelmann, W. K., Digenis, A. G. and Tobin, G. R, "Physiology and healing dynamics of chronic cutaneous wounds," *American Journal of Surgery*, 176 (2). Supplement 1. 26S–38S. Aug. 1998.

[14] Harold, B. and Marjana, T.C, "Cellular and molecular basis of wound healing in diabetes" *J. Clin. Invest*., 117 (5). 1219-1222. May.2007.

[15] Falanga. V, "Wound healing and its impairment in the diabetic foot," *Lancet* 366 (9498) 1736-1743. Nov. 2005.

[16] Maruyama K., et al. "Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing," *Am. J. Pathol.*, 170. 1178-1191. 2007.

[17] Erdinc. K., Haluk, R. U., Omer, G., Gulden, D., Ragip, O., Murat, O., Hayrullah, D. and Mehmet, A.O, "Effect of Ascorbic Acid on Incisional Wound Healing in Streptozotocin-Induced Diabetic Rats," *Wounds*, 22(2).27-31. Feb. 2010.

[18] Satyanarayana, K., Mangathayaru,V., Shreekanth, J., Venkateshwarlu, V. and Kokate, C.K, "Studies on hypoglycemic and cardiotonic effects of roots of Cocculus hirsutus," *J. Pharmaceutical Sci*., 63. 30-35. 2001.