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Original Research Article

Effects of *Moringa oliefera Lam.* Aqueous Root Extract on the Histology of Pancreas in Alloxan-Induced Diabetic Rats

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Abstract

This study was to determine the effects of aqueous root extract of *Moringa oleifera* on antioxidant activities and on the histology of pancreas in Alloxan-induced-diabetic rats. Twenty Albino Wistar rats of both sexes weighing between100 and 194g were used for the study. The animals were divided in to four groups (Groups I, II, II and IV) of five rats each. Group I was the control group and were administered distilled water per body weight. Group II was induced with diabetes by injecting the rats with 150mg/kg Alloxan single dose and not treated. group III was induced with diabetes and treated with 50mg/kg *Moringa* for 28 days and group IV were not induced with diabetes but were treated with the aqueous extract of *Moringa oleifera* root for a period of twenty-eight days at 50mg/kg orally. The antioxidant activity of *Moringa oliefera* aqueous root extract was studied by assaying serum marker substances of Catalase, Glutathione peroxidase (GPx), Superoxide Dismutase (SOD) and Malondialdehyde (MDA) to determine the extent of antioxidant activity of the extract on the pancreas. At the end of the experiment, the animals were sacrificed and the Pancreas processed for routing light microscopic analyses. The results of this study showed that antioxidant activities of Malondialdehyde (MDA) decreased significantly in diabetic not treated and diabetic treated *Moringa* groups and Glutathione peroxidase (GPx), activity increased significantly in diabetic non-treated group and non-diabetic treated group ($p<0.05$). Glutathione Superoxide Dismutase (SOD) and Catalase changes were not significant. The results of this study also showed that photomicrograph of pancreas of diabetic non treated rats showed highly lobulated pancreatic tissue, glands exhibits loose collagenous capsule which can be seen extending as delicate septae, chronic inflammation and degeneration of islets of Langerhans. Diabetic treated group also showed septea between the lobule similar to the control group and glandular acini undergoing regeneration. Non-diabetic but treated group showed diffused micro and macro acini and normal islets of Langerhans similar to control.

Keywords: Alloxan-Induced. Diabetic Rats. *Moringa oliefera*. Pancreas.

INTRODUCTION

Moringa oliefera (*Moringa* pterygosperma Gaertn), a family of *Moringaceae* a perennial angiosperm plants, which have 12 other species (Olson, 2002). *Moringa oliefera* is a native to sub-Himalayan, northern parts of India. It is cultivated throughout tropical and sub-tropical areas of the world and known as Drumstick in English, *Zogalegandi*, in Hausa, *Okweoyibo*in Igbo *and Ewe Igbale*in Yoruba (Sofowora, 1993). Drumstick, Horseradish and Malunggay, are the most common names of Moringa in many parts of the world (Ramachandran *et al*, 1980). The phytochemical analyses of this plant have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A, C and D, essential amino acids, β-carotene, and flavonoids (Bennett *et al*., 2003; Aslam *et al*., 2005). *Moringa oliefera* in many regions of Africa is widely consumed for self-medication by patients affected by diabetes, hypertension, or HIV/AIDS (Dieye *et al*., 2008). In Nigeria people consume *Moringa* leaves because they are rich in vitamins A, B and C, calcium, potassium proteins etc. The leaves, roots, stem back, seed and pods also are used for treatment of malaria, typhoid, antipyretic, weight loss, improvement of cardiovascular system, enhancing fertility and even water purification, roots of the plant is used for cosmetics. There are 425 million people that have diabetes in the world and more than 16 million are in Africa and it has been projected

that by 2045 the number will be around 41 million (IDF, 2006). Nigeria is one of the 32 countries of IDF African region and in 2015, 1.702.900 cases of diabetes were recorded for adult population in Nigeria (IDF, 2006). Treatment option include: diet management, diet with oral hypoglycemic drugs and diet with insulin. Diabetes mellitus (DM) is a metabolic disorder which results from high blood sugar levels over a prolonged period. The term "diabetes" or "to pass through" was first used in 230 BCE by the Greek Apollonius of Memphis (Leonid, 2009). Diabetes Mellitus (DM) is a metabolic disorder of global concern (Yusuf *et al*., 2001; Wild *et al*., 2004). In developing countries like Nigeria, it is a major cause of morbidity and mortality, due to the progressive transition to a lifestyle partly characterized by greater access to dietary calories and less demand for calorie expenditure (Hossain *et al*., 2007; Aje and Miller, 2009).

MATERIALS AND METHODS

Plant Collection and Authentication

Moringa Oleifera root was harvested during the dry season (February) from home-grown garden in Maiduguri, Borno State, Nigeria. The plant was authenticated by a plant taxonomist, Professor S. S. Sanusi of the Department of Biological Sciences, Faculty of Science, and University of Maiduguri Nigeria. A voucher specimen (No.1223) was prepared and deposited at the Herbarium Department Biological Science University of Maiduguri. Two kilograms of the roots were washed and air-dried under the shade for one week.

Plant Extraction

The dried root was pulverized using pestle and mortar. The dried powder (1kg) was subjected to soxhlet extraction using distilled water, as described by Trease and Evans (2002). The percentage yield was determined and the extract was stored at the Human Anatomy Laboratory until use.

Experimental Animals

Albino Wistar rats were obtained from the animal house of the department of Animal Science, University of Jos and kept in the animal house of the Department of Biochemistry, University of Maiduguri for the experiment. They were housed in rubber cages covered with wire-mesh. The animals were fed with pelletized animal feed (Growers mesh vital feed, Jos) and water *ad libitum*. The rats were allowed to acclimatize to the existing climatic condition for 14 days. Twenty adult albino Wistar rats of both sexes weighing between 100 and 190g were used for the study.

Materials

Apparatus used include: Glucometer, test strips, weighing balance, photomicroscope, light microscope, syringes, needles, feeders, cages, specimen bottles, test tubes, slides, cover slips, dissecting kits and beddings. Laboratory Reagents used include; Alloxan monohydrate (manufactured by Krishna chem. Industry Vadodara Sayajiganj, India), haematoxylin and eosin (H&E), alcohol, xylene, distilled water, paraffin wax, formalin and EDTA.

Induction of Diabetes in Rats

Diabetes was induced using slow intraperitoneal injection of 1% solution of Alloxan at 150mg/kg body weight dissolved in distilled water after fasting the animals overnight, and administered within few minutes of its preparation. Diabetes was confirmed on the fourth day by blood glucose determination device (glucometer/test strips) (Bharali *et al.*, 2003; Das *et al*, 2012).

Body Weight Determination and Dose Administration

Animals were marked, weighed using the digital weighing balance and grouped randomly. The animals were divided into four groups (I, II III and IV) of 5 rats each and the weights of animals were recorded. Doses of Alloxan and *Moringa oleifera* were administered based per body weights of the rats.

Experimental Design

A total of 20 Wistar albino rats were used for the study. After acclimatization of the animals for 14days, the rats were divided into 4 groups of 5 rats each. The rats were grouped as follows:

Group I was the control group in which the rats received only the vehicle (distilled water) in equivalent dose volume per body weight.

Group II (diabetic non-treated) consist of 5 rats which were induced with diabetes by injecting 150mg/kg Alloxan monohydrate solution after fasting the animals overnight. The animals also received distilled water per body weight. *Moringa* extract was not given to this group. Those rats with blood glucose level of 7mmol/l and above were considered diabetic.

Group III (diabetic treated) composed of 5 rats induced with diabetes (150mg/kg) and treated with 50mg/kg *Moringa oliefera* aqueous root extract.

Group IV (non-diabetic treated) consisted of 5 rats that was not induced with diabetes but received *Moringa oleifera* aqueous root extract 50mg/kg body weight. All treatments commenced on the 4th day of the induction of diabetes and lasted for 28 days.

Animal Sacrifice, Blood Sample Collection and Tissue Procurement

Animals were sacrificed by injecting the animals with ketamine (anesthesia) 120mg/kg and the blood samples were collected by cardiac puncture into

sterilized EDTA specimen bottles for antioxidant activity analyses. Antero- median incision was made on the abdominal wall of the Wistar rats for the removal of the whole pancreas lying inferior to the stomach and spleen and attached to the center of the curvature of the duodenum.

Antioxidant Activity Analyses

Antioxidant activities analyses were carried out at the Histochemistry laboratory of the Kwara State University (KWASU): Assay of Catalase activity was evaluated according to the method described by Aebi (1984). The activity of catalase was expressed as unit mg^{-1} of protein. Assay of Superoxide dismutase activity was evaluated according to the method described by Winterbourn *et al*, (1975). It was expressed as U/mg^{-1} protein. Assay of Glutathione activity was determined according to the method described by Rotruck *et al*, (1973). The absorbance was read at 400nm and it was expressed as nmol-protein. Assay of Malondialdehyde (MOD): Lipid peroxidation was measured colorimetrically by thiobarbituric acid reactive substance method described by Buege and Aust (1978). Concentration was estimated using the molar absorptive of Malondialdehyde which is $1.56x105m^{-1}$ cm and it was expressed as nmol mg^{-1} protein.

Assessment of Effects on Histology of Pancreas

Pancreatic tissues were harvested and fixed in neutral buffered formalin for 48hours. The tissues were trimmed, dehydrated in ascending graded series of alcohol (30%, 50%, 80%, 95% and 100%). The tissues were cleared in xylene, embedded in paraffin wax, sectioned between 3 to 5µ and stained with hematoxylin and eosin. Photomicrograph of the tissues were taken using photomicroscope (Olympus C-5A, Tokyo Japan 203250) at x100, x200 and x400 magnifications (Das *et al*, 2012).

STATISTICAL ANALYSIS

Data obtained from this study were analyzed to determine the differences between and within groups for antioxidant activity. One-way analysis of variance (ANOVA) was calculated followed by Bonferoni Post Hoc test using statistical package for social sciences (SPSS) version 21. Values were presented as Mean ± SD and levels of significant for differences observed was atp<0.05.

Moringa groups at P<0.05. Glutathione Peroxidase (GPx) activity increased significantly in diabetic non-treated group and non-diabetic but treated group (p<0.05). Superoxide Dismutase (SOD) and Catalase were not significant as Oxidative stress markers in the experimental animals as shown in Table 1.

RESULTS

Antioxidant Activities of *Moringa oleifera* Root Aqueous Extract

Antioxidant activities showed that Malondialdehyde (MDA) activity decreased significantly in diabetic not treated and diabetic treated

Table-1: Effects of *Moringa Oleifera* Aqueous Root Extract on Oxidative Tests in Alloxan-Induced Diabetic Wistar Rats

Groups	SOD (µ/mL)	MDA (µ/L)	GPx (µ/mL)	CATALASE mU/mL
Control I	8.80±3.59[a]	3.17±2.17[a]	59.50±22.35[a]	0.09±0.04[a]
II (D N T)	10.28±0.45[a]	0.64±0.28[b]	85.34±21.75[b]	0.01±0.90[a]
III (D T M)	9.92±0.43[a]	0.75±0.15[b]	57.72±11.97[a]	0.20±0.01[a]
IV (N D T M)	9.88±1.20[a]	2.00±2.70[a]	72.74±38.65[b]	0.02±0.02[a]

Similar superscript indicates no significant difference with control. Values are Mean ± SD*= p≤0.05 (Comparison Relative to Control). (n=5).

Key:

Group I (Control)
Group II (Diabetic non-treated)
Group III (Diabetic but treated with the Moringa root aqueous extract)
Group IV (Non-diabetic but treated with the Moringa root aqueous extract)

Effect of *Moringa oleifera* Aqueous Root Extract on Diabetic and Non-Diabetic Pancreas

Photomicrograph of pancreas of diabetic non treated rats showed highly lobulated pancreatic tissue, the gland exhibits loose collagenous capsule which can be seen extending as delicate septae, chronic inflammation and degeneration of islets of Langerhans was also seen (Figure 1B) when compared with the control group photomicrograph. Diabetic and treated group also showed septae between the lobule similar to control and some glandular acini undergoing regeneration (Figure 2C). Non-diabetic but treated group showed diffused micro and macro acini and normal islets of Langerhans similar to control (Figure 3D).

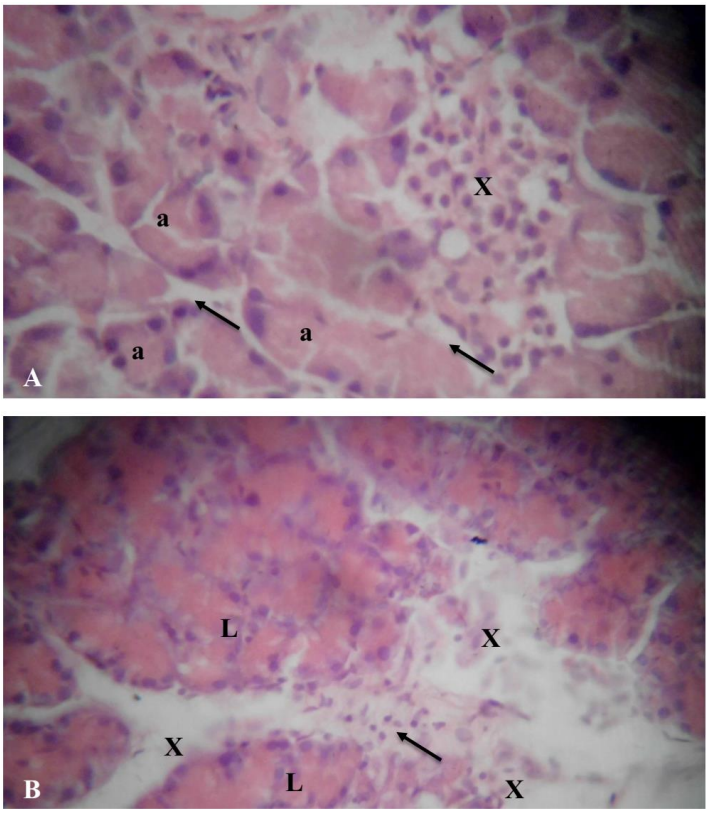

Fig-1: Photomicrographs of (a) control rat pancreas showing remarkable septa (black arrows) islets of Langerhans (X) and glandular acini (A) are normal H&E x400. (B) diabetic non-treated rat pancreas, showing pancreatic tissue highly lobulated (L), gland exhibiting loose collagenous capsule which extends as delicate septa with chronic inflammation (X) and degeneration of islets of Langerhans (arrow) H&E x400.

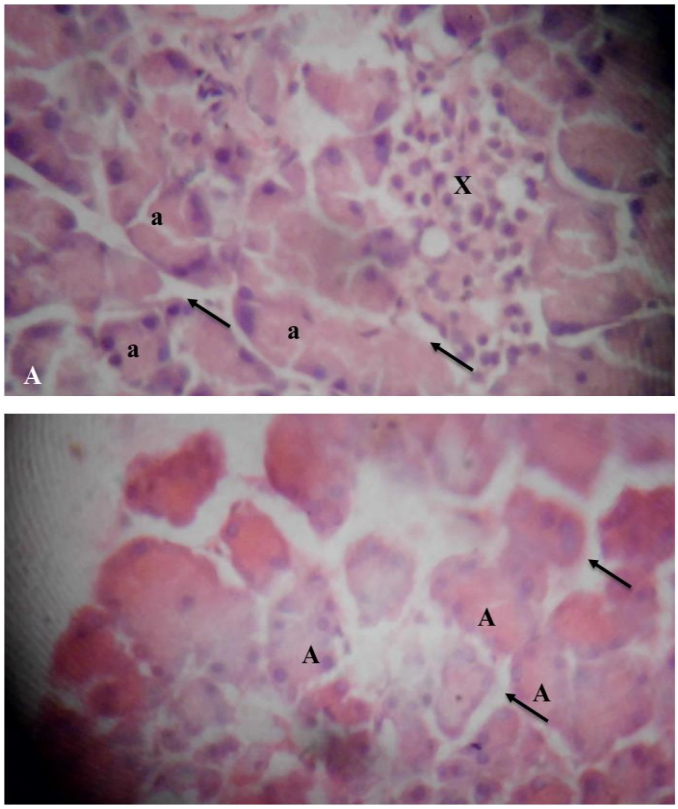

Fig-2: Photomicrographs of (A) control rat pancreas showing remarkable septa (black arrows) islets of Langerhans (X) and glandular acini (A) are normal H&E x400. (C) Diabetic treated rat pancreas showing clear septae between the lobules similar to control (arrows), glandular acini undergoing regeneration (A) H&E x400.

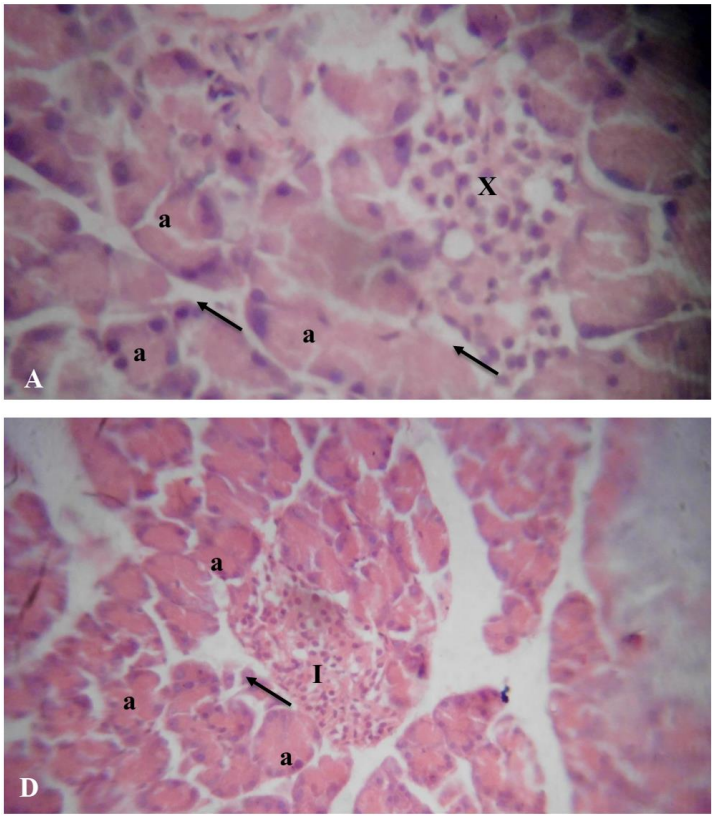

Fig-3: Photomicrographs (A) control rat pancreas showing remarkable septa (black arrows), islets of Langerhans (X) and glandular acini (A) are normal H&E x400. (D) Non-diabetic but treated rat pancreas showing diffuse micro (black arrow) and macro acini (a) normal islet of Langerhans (I) other features as in control H&E x400.

DISCUSSION

Diabetes mellitus is one of the leading causes of morbidity and mortality in the world. Its prevalence rate has continuously increased globally (WHO, 2014). Alloxan-induced diabetes is one of the widely used methods of inducing type 1 diabetes mellitus in experimental animals (Rohilla and Ali, 2012). This is because Alloxan has been found to be selectively toxic to the beta cells of the pancreas which are responsible for insulin synthesis (Goldner and Gomori, 1944). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (Dieye, *et al*, 2008). The current study also investigated the lipid Peroxidation in the blood samples by observing the concentrations of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Malondialdehyde (MDA) as oxidative markers in the test animals as compared to the control group. This was aimed at identifying the oxidative effect of Alloxan on the blood samples and the consequent effect of diabetes on the pancreatic architecture in the Albino rats. The raised values observed in GPx and the decreased also observed in MDA might be as a result of the damage caused by both the Alloxan substance and the diabetic condition on the pancreatic beta cells and pancreatic cells in the experimental animals. Histopathological observations of the pancreatic tissue photomicrograph suggested that the reactive oxygen species and lipid peroxidation may play an important role in pathogenesis of pancreatic beta cells that led to the loss of the normal architecture of the beta cells and development of diabetes mellitus. Because of Alloxan toxicity, toxic reactive metabolites superoxide free radicals were produced which bound covalently to macromolecules of the lipid membranes of the beta cells and caused peroxidative degradation of the affected cells. The degenerative changes were shown to be minimal with the plant extract treatment among the diabetic treated group. This might be due to lower fat accumulation and re-establishment of the antioxidant defense system in the pancreas through the antioxidant and antidiabetic nature of *Moringa oleifera* aqueous root extract as compared to the control and the diabetic non-treated groups.

On the histology of the pancreas, the photomicrographs of the control rat pancreas when compared with the various treatment groups indicated that the diabetic non treated rats showed highly lobulated pancreatic tissue, gland exhibiting loose collagenous capsule which extend as delicate septae with chronic inflammation and degeneration of islets of Langerhans. Diabetic treated group Showed clear septae between the lobule similar to control and glandular acini undergoing regeneration. Non-diabetic treated group showed diffused micro and macro acini and normal islets of Langerhans similar to control.

CONCLUSION

This study concludes that *Moringa oleifera Lam*. aqueous root extract has anti-diabetic property. It also relatively reverses the damage caused by Alloxan monohydrate as seen in regeneration of some of the islets of Langerhans and acini of the pancreas.

REFERENCES

- Aebi, H. (1984). Catalase. Methods enzymatic Analysis, Bergmeyer H. Ed. Verlag chemical. *Weinhelm*, *3*; 273.
- Aje, T. O., & Miller, M. (2009). Cardiovascular disease: A global problem extending into the developing world. *World Journal of Cardiology, 1*; 3– 9.
- Aslam, M., Anwar, F. and Nadeem, M. (2005). Mineral composition of *moringa Oliefera* leaves and pods from different regions of Punjab, Pakistan: *Asian Journal of Plant Science*, 4: 417–421.
- Bennett, R. N., Mellon F. A., & Kroon P. A. (2003). Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera: (Horseradish tree) and *Moringa stenopetala* L: *Journal of Agricultural Food Chemicals*, 51; 200-210.
- Bharali, R. Tabassum, J. Azad, M. (2003). Chemomodulatory effect of *Moringa oleifera* Lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice: *Asia Pec. J. Cancer Pre*v., 4: 131-139.
- Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. Methods of Assay mology, New York. Academic press 302-310.
- Das, J., Van, V., & Sil, P. C. (2012). Taurine exerts hypoglycemic effectin alloxan-induced diabetic rats, improves insulin -mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress apoptosis. *Toxoid Appl Pharmacol* 258:296-308.
- Dieye, A., M., Sarr, A., & Faye, B. (2008). Medicinal plants and the treatment of diabetes in Senegal Survey with patients*: Fundamental of Clinical Pharmacology*. 22:221-228.
- Goldner, M. G., & Gomori, G. (1944). Studies on the mechanism of alloxan diabetes: Endocrinology, 35:241-248.
- Hossain, P., Kawar, B., & El-Nahas, M. (2007). Obesity and diabetes in the developing world: A growing challenge. *New England Journal of Medicine*.356: 213–217.
- IDF, International Diabetes Federation (2006). *Diabetes Blue CircleP.7*
- Leonid, P. (2009). Principles of diabetes mellitus. 2nd Ed. *New York*. Pp. 25.
- Olson, M. E. (2002). Combining data from DNA sequences and morphology for a phylogeny of Moringaceae: *Systemic Botany*, 27; 55-73.
- Ramachandran, C., Peter, K. V. and Gopalakrishnan, P. K. (1980). Moringa oleifera, a multipurpose Indian vegetable: *Economic Botany*.34: 276-283.
- Rohilla, A., & Ali, S. (2012). Alloxan-induced diabetes: Mechanisms and effects. Int. J. Pharm. Biomed. Sci.
- Rotruck, T. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G. and Hoekstra, W. G. (1973). Selenena. Biochemical role as a component of glutathione peroxidase. *Sci*. 179-588-590.
- Sofowora, A. (1993). Medicinal plants and traditional medicinein Africa. Spectrum Books ltd. Ibadan, Nigeria. Pp.289
- Trease, G. E., & Evans, W. C. (2002). Textbook of pharmacognosy. Phytochemical extraction. 14th ed. pp. 13-53.
- WHO, World Health Organization. (2014). Diabetes fact sheet. No. 312.
- Wild, S., & Roglic A. (2004). Global prevalence of diabetes: estimate for the year 2000 and projection for 2030. *Diabetes Care, 27*(104): 1047-1053.
- Winter Bourn, C. C., Hawkins, R. E., Brain, M., & Canell, R. W. (1975). The estimation of red cell superoxide dismutase activity. *J. lab. Clin. Med*. 85: 85 337-341.
- Yusuf, S., Reddy, S., & Anand, S. (2001). Global burden of cardiovascular diseases: Part II: Variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies.