Anti-diabetic Effect of Neem and Spirulina in Alloxan Induced Diabetic Mice

Fatema Akter[1], Mohammad Mahmudur Rahman[1*], Mahbub Mostofa[1]and Emdadul Haque Chowdhury[2]

[1]Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

*Corresponding author

KEYWORDS	A B S T R A C T
Diabetes mellitus; mice; Neem; Spirulina; alloxan.	In this study the anti-diabetic effects of neem and spirulina were evaluated in alloxan-induced diabetic mice. After acclimatization, 25 Swiss Albino male mice *(Mus musculus)* were randomly divided into five equal groups, from which one was kept as control (C) and other 4 groups of mice were induced diabetes by single intraperitoneal injection of alloxan @ 240 mg/kg body wt. For therapeutic justification, out of 4 alloxan-induced diabetic groups, one was kept as diabetic control (DC) whereas other three groups were treated with oral administration of 500 mg neem leaves/kg body wt (DN), 20 mg/kg body wt spirulina (DS) and combination of neem extract plus spirulina (DNS), respectively, for 42 consecutive days. All three treated groups of mice such as DN, DS and DNS significantly (P<0.001) reduced the up regulated blood glucose, total plasma cholesterol such as LDL and triglyceride (TG). Besides these, the mice of group DN and DNS showed significant *(P<0.001)* increase of erythrocytes and decrease (P<0.05) of total leukocyte count. The highest body weight gain was found in the mice of group DN (P<0.05). We, therefore, conclude that crude extract of neem leaves and/or spirulina could be an effective alternative therapy for diabetic patients.

Introduction

Diabetes mellitus, a metabolic disorder, is caused either by the insufficient production of insulin or the inability of the body to respond to the insulin formed within the system. It is a major health problem and one of the leading causes of death in humans and few animals especially in the dog (Adams, 1995). Recent record

indicates that in 2010, an estimated 285 million people had diabetes of which type 2 were about 90% of the cases (Melmed *et al.,* 2012). Its incidence is increasing rapidly, and by 2030, this number is estimated to be almost double (Wild *et al.,* 2004). According to the latest WHO data published in April 2011 deaths due to diabetes mellitus in Bangladesh reached 19,598 or 2.05% of total deaths.

For proper investigation and research, experimental animal models play a pivotal role for therapeutic efficacy of candidate drug. Experimental diabetes (ED) can be induced by pancreatectomy, administration of insulin-antagonist hormones or other chemical agents. There are three groups of chemical agents used to induce ED, the first group destroys the beta cells of the pancreatic islets; the second group alters the beta cells but do not destroy them and the third group increases the endogenous insulin requirements weakening the pancreas and producing ED (Mendez *et al.,* 1994). Streptozotocin (STZ) and alloxan are the chemical inducers of ED mostly used in laboratory animals. STZ is a methylating agent for DNA (Bennett *et al.,* 1981) that destroys pancreatic beta cells, inducing permanent diabetes. Alloxan is a toxic agent for pancreas beta cells; its proposed mechanism for diabetes induction includes: sulfhydryl group attack, chelant action, enzyme and metabolic modifications; membrane transport changes on electrolytes (Carrol *et al.,* 1994) plus increased lipoperoxidation (Soto *et al.,* 1994). In our study we used alloxan to induce diabetes in Swiss albino mice.

Two groups of drugs are commonly used in the treatment of diabetes: insulin and its preparations and oral hypoglycemic drugs i.e. glibenclamide, tolbutamide, glipizide, metformin, phenformin and glycodiazine.

Although there are a number of drugs available on the market, long time use may cause a number of side effects. Hence a large number of studies are in progress to find natural sources, which are effective in reducing the intensity of diabetes. More than 13,000 plants have been studied during the last 5 year period. Indian, Egyptian and Greek physicians were the first who described diabetes as a disease long before modern drugs became available to treat the condition. In the most part of the world anti-diabetic medicinal plants were used to treat diabetes before the early 1920s when the blood sugar regulating compound insulin was discovered and in more traditional cultures, natural plant products were the choice of treatment for diabetes (Roberts, 2001).

Neem has been used as traditional remedies for treatment of various forms of diseases from antiquity. All parts of the plant are said to have some medicinal properties (Biswas *et al.,* 2002). It is traditionally used for treatment of arthritis, leprosy, typhoid, respiratory disorders, constipation, chronic fatigue, cancer, chronic syphilis sores and indolent ulcer. It is also traditionally used as tonic and astringent for wounds, tooth decay and gum diseases (Biswas *et al.,* 2002) and as a general health conditioner it is said to be a potent antimalarial, antifungal and antibacterial agent (Van der Nat *et al.,* 1986). Whereas, spirulina contains a wide spectrum of nutrients that include B-complex vitamins, minerals, trace elements, good quality proteins, gamma-linolenic acid and the super antioxidants, beta carotene, vitamin E, phycocyanine and chlorophyll (Layam *et al.,* 2006).

Indiscriminate information is available regarding the effects of neem and spirulina on alloxan induced diabetic mice.

Therefore, considering the above mentioned background we have investigated the therapeutic effects of neem leave extract and commercially available spirulina on body weight, blood glucose level, lipid profile and hematological parameters of alloxan induced diabetic mice.

Materials and Methods

Experiments were carried out at the Department of Pharmacology, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Completely Randomized Design (CRD) was conducted in this work. All works were performed according to the ethics of laboratory animal use.

Experimental animals

One month old twenty five (25) healthy adult male Swiss albino mice were collected from International Centre for Diarrhoeal Diseases Research and Rehabilitation, Bangladesh (ICDDR,B) Mohakhali, Dhaka. They were maintained in controlled environmental conditions of temperature and humidity on alternative 12 hr light/dark cycles. All animals were fed standard mice pellet (ICDDR,B) and water *ad libitum*. All mice were maintained in the animal care facility according to university animal care and use guidelines.

Neem Leaf Extract (NLE) and Spirulina

10% aqueous solution of neem leaf extract was prepared in our laboratory. Pharmacological doses were maintained by oral administration of 500 mg leave extract/ kg/ day for 42 days treatment period. Spirulina was purchased as commercially available Spirulina M® (BCSIR, Science Laboratory, Dhaka, Bangladesh). Spirulina

was administered @ 20 mg/ kg body weight orally for consecutive 42 days.

Induction of Diabetes and Treatment

After one week of acclimatization period all the mice were randomly divided into 5 equal groups; each group consisting 5 mice. The groups were designated as follows: Out of five groups, one group of mice was kept as control (C) without giving any drugs except vehicle and other 4 groups of mice were induced diabetes by intraperitoneal (ip) injection of alloxan (Sigma- Aldrich, UK) @ 240 mg/kg body wt as a single injection. For therapeutic justification, out of 4 alloxan-induced diabetic groups of mice, one group was kept as diabetic control (DC) and the other three groups were treated with oral administration of 500 mg neem leaves/kg bd wt as 10% aqueous extract named Diabetic Neem (DN), 20 mg/kg body wt spirulina called Diabetic Spirulina (DS) and combination of 10% aqueous neem extract plus 20 mg spirulina /kg bd wt named as Diabetic Neem and Spirulina (DNS), respectively for 42 consecutive days.

At the end of the experimental period (42 days), the mice were euthanized by carbon dioxide asphyxiation and blood was withdrawn by cardiac puncture using a 22 gauge needle and a 5 mL syringe. About 1mL of blood from the syringe was taken in the test tube containing anticoagulant (3.8% sodium citrate solution) for hematological studies and 0.5 mL was collected without anticoagulant for serum analysis of lipid profiles.

Determination of Body Weight

Body weight of all the treated groups (4 groups) and control group of mice were recorded before treatment (on day 0) and

during treatment period i.e. 7th, 14th, 21st, 28th, 35th and 42nd day. A properly calibrated and standardized electronic balance was used for taking body weight of the mice and expressed as gram (g).

Determination of Blood Glucose

Blood samples were collected from tail vein of mice on day 0 (pre-treatment), 21 and 42 (during treatment) for estimation of blood glucose using commercially available glucometer (Kare Blood Glucose Test Strip) and results were demonstrated in mmol/ L.

Biochemical Analysis

Serum total cholesterol, triglyceride and high density lipoprotein (HDL) were assayed by conventional enzymatic methods on a Hitachi 911 automated analyzer from Roche Diagnostics (Laval, QC, Canada) using the procedure described by Trinder (1969). Total cholesterol was determined using CHOD-PAP method. The precision performance of these assays was within the manufacturer's specifications. LDL cholesterol was calculated by the Friedewald equation (Friedewald et al., 1972). All the results were expressed in mg/dL.

Hematology

Total erythrocyte count (TEC), hemoglobin contents (Hb) and total leukocytes count (TLC) were carried out according to the methods described by Lamberg and Rothstein (1977).

Statistical analysis

All data were expressed as mean ± SEM and differences among the groups of animals were compared using one-way ANOVA with post-hoc LSD and Duncan's test. Statistical significance was set at $P< 0.05$. Statistical analysis was performed using SPSS software version 17 (SPSS Inc., Chicago, IL, USA).

Result and Discussion

Effects of Neem and Spirulina on Body Weight

The result of effect of neem leaves extract and spirulina on body weight of alloxan induced diabetic mice is shown in Figure 1. Mice at day 0 were found in and around 25 gm each. In the first week both diabetic non-treated and treated mice such as 10% neem leaf extract (DN); 20 mg/kg body wt spirulina (DS) and 10% neem leaf extract plus 20 mg/kg body wt spirulina (DNS), respectively, were started to reduce body weight. However, from second week, diabetic treated mice started to improve body weight, whereas, diabetic non-treated (DC) mice gradually decreased body weight during the experimental period (Figure 1)

This result has similarities with the findings of Bopanna *et al.,* 1997; Akpan *et al.,* 2012 who found that body weight of all the treated groups were significantly ($P<0.05$) increased with neem treatment compared to diabetic mice. They suggested that this may be due to some constituents of the neem extract which may have mimicked or stimulated the actions of growth factors hence its ability to enhance the repair and regeneration of damaged pancreatic tissue. On the other hand, spirulina also increased body weight significantly ($P<0.05$) which was also reported by Maged *et al.,* (2004) in diabetic rats. However, diabetic rats treated with SM showed increase in body weight may be explained by increased

insulin secretion or increased food consumption (Pandey *et al.,* 2011).

Effects of neem and spirulina on blood glucose level

Mice that were treated with alloxan alone (DC) remarkably showed high blood glucose level, whereas, mice treated with 10% neem leaf extract (DN); 20 mg/kg body wt spirulina (DS) and 10% neem leaf extract plus 20 mg/kg body wt spirulina (DNS) significantly (P<0.001) reduced blood glucose levels respectively (Figure 2). It is reported that the hyperglycemic effect of alloxan is may be due to damage the β cells of pancreas that interfered the synthesis of insulin which might be responsible for the metabolism of glucose (Bopanna *et al.,* 1997).

However, treatment with neem or spirulina alone or their combination remarkably protected the up regulation of blood glucose level. Neem treatment significantly (*P*<0.001) reduced blood glucose level which was also reported by other scientists (Bopanna *et al.,*1997; Kholsa *et al.,* 2000; Chattopadhyay *et al.,* 1993; Chattopadhyay 1999). The mechanism of the antidiabetic properties of the extract is not well known. Jelodar *et al.,* (2005) had suggested that the antidiabetic properties of the extract may be related to the ability of the extract to stimulate sufficient production of insulin by the pancreas, that aided in the peripheral utilization of glucose in the cells or a possible ability of the extract to regenerate the β cells to carry out its functions.

On the other hand, spirulina also showed a similar effect by significantly (*P*<0.001) lowering blood glucose level which was reported by Rodriguez-Hernandez *et al.,* (2001) as the administration of 5% *Spirulina maxima* in the diet to normal or diabetic animals decreased blood glucose level significantly (*P*<0.01) in male mice. The possible mechanism by which spirulina brings about its antihyperglycemic action may be through potentiating the pancreatic secretion of insulin from islet β-cell or due to enhanced transport of blood glucose to the peripheral tissue (Anuradha and Vidhya, 2001; Mani *et al.,* 2000). But their combined preparation is not used yet. This is the first report and found that, neem and spirulina combined preparation also reduced blood glucose level significantly (*P*<0.001) close to control mice compared to neem and spirulina alone.

Effects of neem and spirulina on lipid profile

We found that, total cholesterol level was raised significantly (P<0.001) in diabetic mice (DC). On the contrary, up regulated total cholesterol was significantly (P<0.05) decreased in mice of group DN, DS and DNS (Figure 3). We then examined serum triglyceride profile and found that alloxan induced diabetic mice (DC) significantly (P<0.001) increased compared to control mice (Figure 3). After treating them with neem or spirulina or their combination triglyceride level was also significantly (P<0.001) decreased. In addition, neem and neem plus spirulina also increased (P<0.001 and P<0.01) plasma HDL level and decreased LDL (P<0.001) respectively in our experiment.

In alloxan induced diabetic mice the content of total cholesterol increased significantly (*P*<0.001) in all groups (Bopanna *et al.,* 1997). This may be due to excess of fatty acid in plasma produced by the alloxan induced hyperglycemia promotes the liver for conversion of some fatty acids into phospholipids and cholesterol. These two substances along

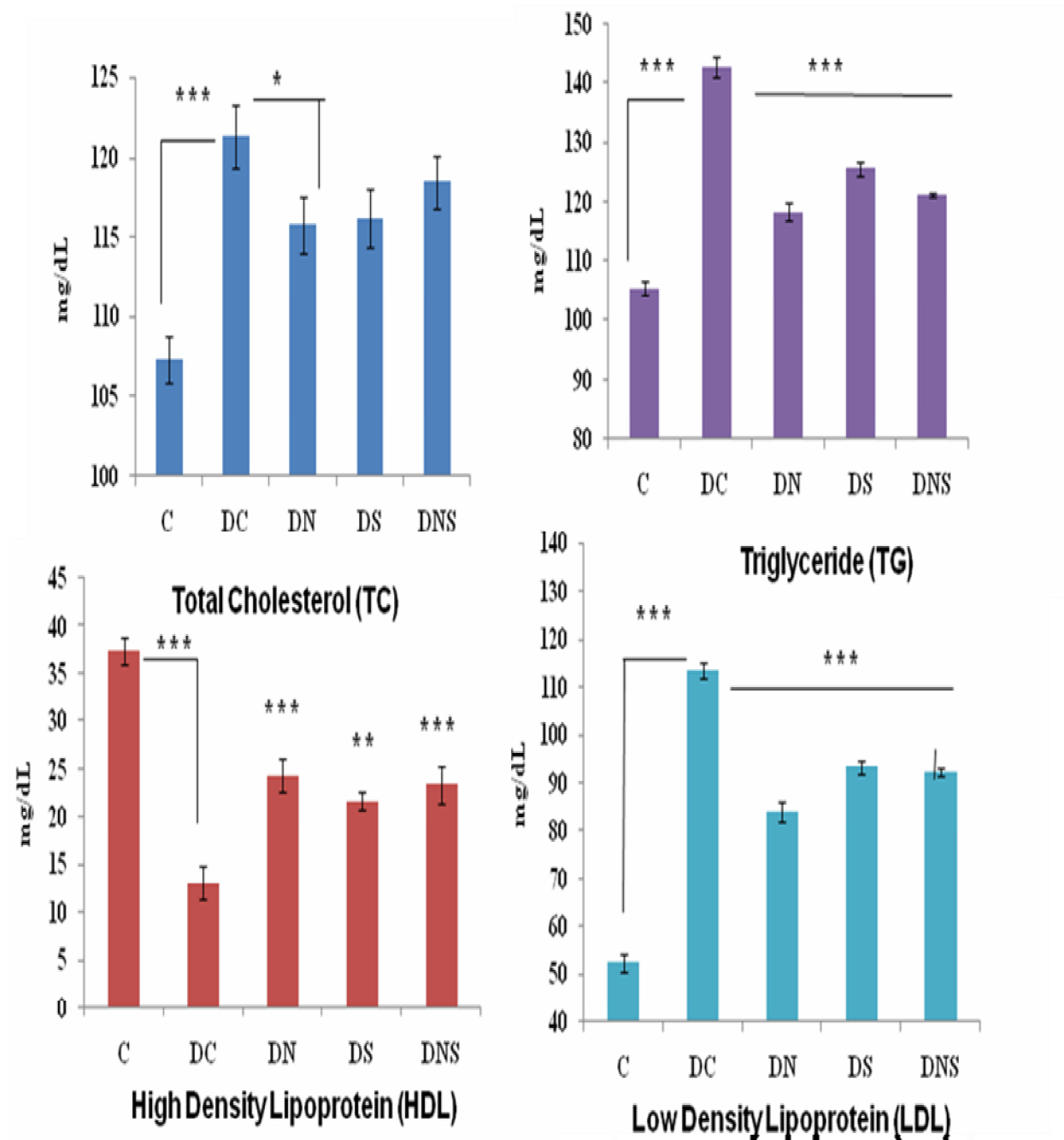**Figure.1** Effects of neem and spirulina on body weight (g) in alloxan-induced diabetes mice.
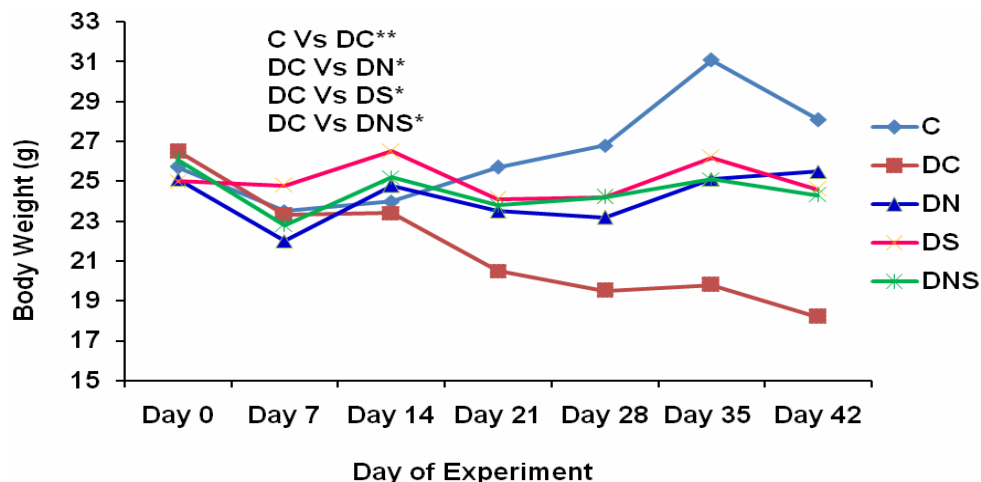
Results are expressed as mean ± SEM. n=5. Values with different superscripts differ significantly (*P*<0.001); ***** = Significant at 5 percent level (*P*<0.05); ****** = Significant at 1 percent level (*P*<0.01); ******* = Significant at 0.1 percent level (*P*<0.001) C, control; DC, diabetic Control; DN, diabetic + neem; DS, diabetic + Spirulina; DNS, diabetic + Neem + Spirulina

**Figure.2** Effects of Neem and Spirulina on blood glucose (mmol/L) in control and diabetic mice
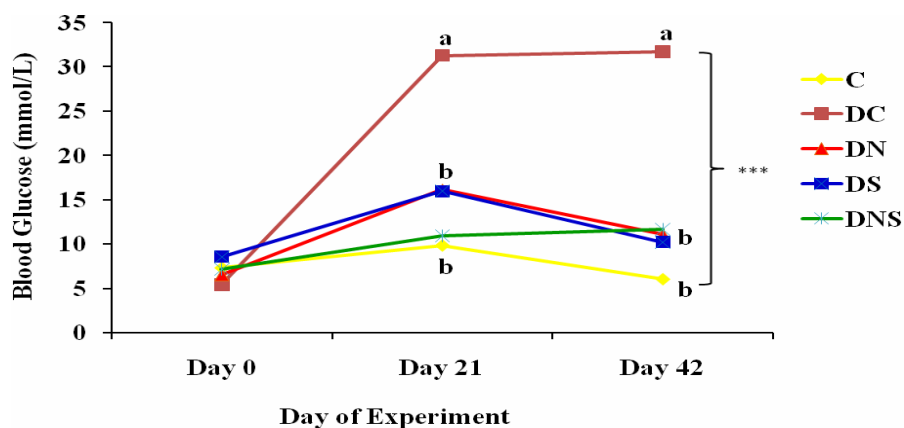
Results are expressed as mean ± SEM. n=5. Values with different superscripts differ significantly (*P*<0.001); ***** = Significant at 5 percent level (*P*<0.05); ****** = Significant at 1 percent level (*P*<0.01); ******* = Significant at 0.1 percent level (*P*<0.001) C, control; DC, diabetic Control; DN, diabetic + neem; DS, diabetic + Spirulina; DNS, diabetic + Neem + Spirulina.

Figure.3 Effects of neem and spirulina on lipid profile in alloxan-induced diabetes mice

Results are expressed as mean±SEM. n=3. *, P<0.05 and **, P<0.01 respectively significant with the diabetes control (DC) and all treated groups. C, Control; DC, Diabetic Control; DN, Diabetic + Neem; DS, Diabetic + Spirulina; DNS, Diabetic + Neem + Spirulina.

Figure.4 Effects of neem and spirulina on blood profile in alloxan-induced diabetes mice
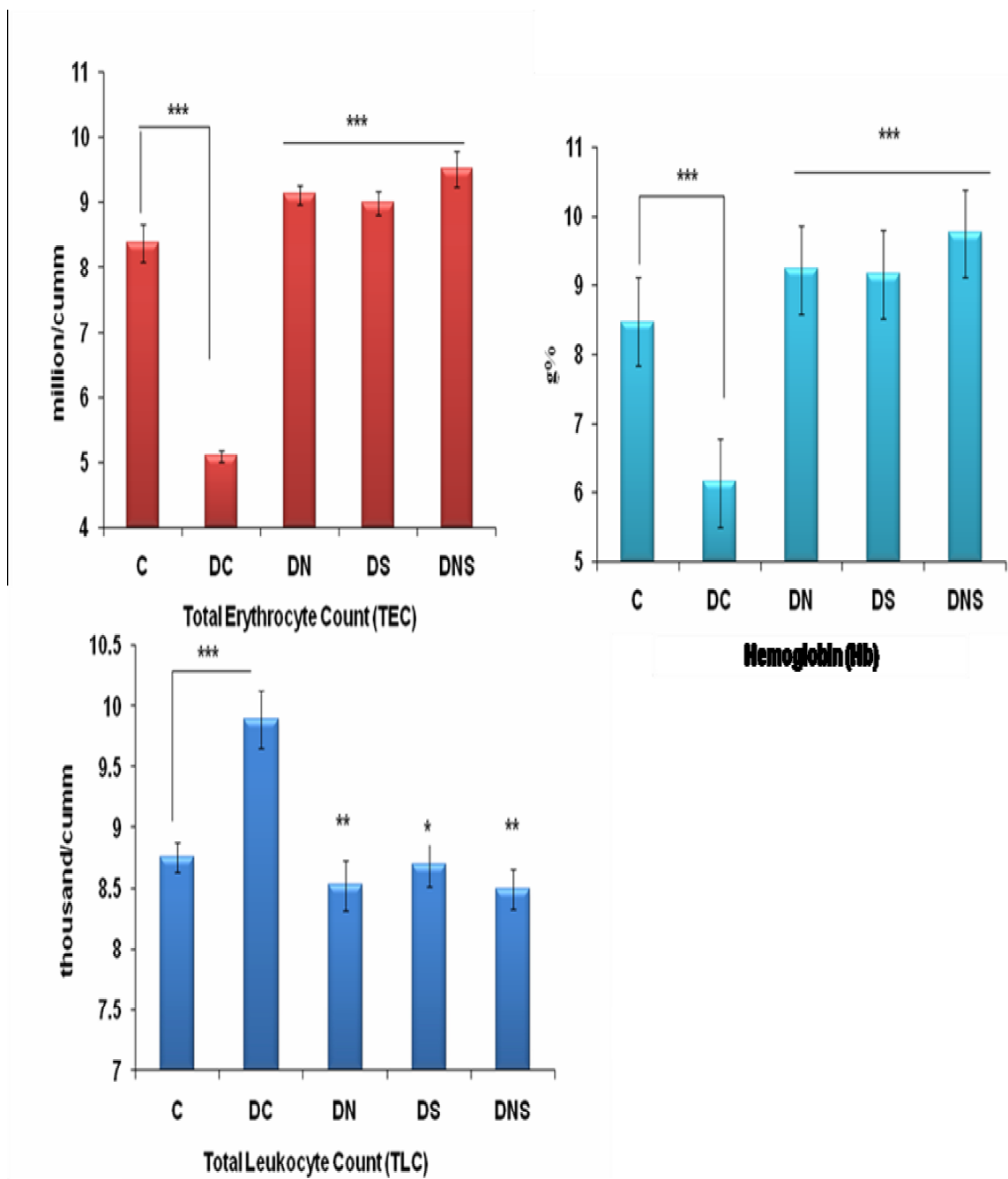
Results are expressed as mean±SEM. n=5. *, $P<0.05$,**, $P<0.01$ and ***, $P<0.001$ respectively significant with the diabetes control (DC) and all treated groups. C, Control; DC, Diabetic Control; DN, Diabetic + Neem; DS, Diabetic + Spirulina; DNS, Diabetic + Neem + Spirulina.

with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins (Bopanna *et al.,* 1997). Following the administration of neem and spirulina the serum total cholesterol level was significantly reduced (*P<0.05*) in neem treatment. There was no significant change between the effects of spirulina and its combination with neem. *Azadirachta indica* (neem) treatment caused significant lowering of serum lipids, decreased the formation of lipid peroxides estimated as thiobarbituric acid reactive substance (TBARS) and increased antioxidants such as superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase in erythrocytes (Halim 2003). The diabetic mice increased triglyceride level, whereas, diabetic mice treated with neem, spirulina and their combination decreased the triglyceride significantly (*P<0.001*) (Anitha *et al.,* 2010).

Iwata *et al.* (1990) observed that spirulina supplementation inhibited the increase of HDL - cholesterol, triglycerides, and phospholipids in the plasma. On the other hand, there was no statistical significance observed between the control group and the groups supplemented with spirulina when lipid levels in the liver were compared. Furthermore, the authors reported an increase in lipoprotein lipase enzyme activity in the animals that received spirulina supplementation.

The results found in the literature on the relationship between lipid profiles and spirulina intake need more controlled studies. Recently Cheong et al. (2010) affirmed that the anti-hypercholesterolaemia mechanisms of spirulina are still not well understood, although some authors suggest that the addition of this alga into the diet diminishes the intestinal absorption of cholesterol as well as the re-absorption of bile acids in the ileum. Thus, they suggest that spirulina can be considered a functional food capable of reducing the levels of cholesterol and consequently preventing diabetes mellitus.

Effects of neem and spirulina on hematological parameters

Induction of diabetes by alloxan in mice, TEC and hemoglobin content were significantly (P<0.001) reduced compared to control (C) group. However, by treatment with neem and spirulina both TEC and hemoglobin were significantly (P<0.001) increased than diabetic control mice. On the contrary, alloxan-induced diabetic mice showed significantly (P<0.05) increased TLC compared to control mice but not the treated mice (Figure 4). These results indicated that these plants product may have positive stimulating effects on bone marrow. Itemobong *et al.* (2010) reported that *Azadiracta indica* treatment improved RBC level from diabetic reduced state. Neem, spirulina and their combination are proved to be equally active in increasing the hemoglobin content and maintaining TLC levels in alloxan induced diabetic mice.

Conclusion

Overall, crude extract of neem leaves or spirulina supplementation demonstrated some significant positive effects on alloxan induced diabetic mice. Therefore, neem and/or spirulina could be an effective alternative therapy for diabetic patients.

Acknowledgement

References

Adams, H.R. 1995. Veterinary Pharmacology and Therapeutics, 7th edition. Iowa State University. Pp. 644-652.

Akpan, H.D., Ekaidem, I.S., Usoh, I.F., Ebong, P.E. and Isong, N.B. 2012. Effect of Aqueous Extract of Azadirachta indica (Neem) Leaves on Some Indices of Pancreatic Function in Alloxan-induced Diabetic Wistar Rats. Pharmacologia. 3(9): 420-425.

Anitha, L., and Chandralekha, K. 2010. Effect of Supplementation of Spirulia on Blood Glucose, Glucosylated Hemoglobin and Lipid Profile of Male Non-insulin Dependent Diabetes. Asian Journal of Experimental Biological Sciences. 1(1): 36-46.

Anuradha, V., and Vidhya, D. 2001. Impact of administration of spirulina on the blood glucose levels of selected diabetic patients. Indian Journal of Nutrition and Dietetics. 38: 40-44.

Bansal, R., Ahmad, N., and Kidwai, J.R. 1980. Alloxan glucose interaction: effect of incorporation of 14C-leucine into pancreatic islets of rats. Acta diabetologicalatina. 17: 135-143.

Bennett, R.A., and Pegg, A.E. 1981. Alkylation of DNA on the rat tissues following administration of streptozotocin. Cancer Research. 41(7): 2786-2790.

Biswas, K., Chattopadhyay, I., Banerjee, R.K., and Bandyopadhyay, U. 2002. Biological activities and medicinal properties of Neem (Azadirachta indica). Current Science. 82: 1336-1345.

Bopanna, K.N., Kannan, J., Sushma, G., Balaraman, R., and Rathod, S.P. 1997. Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. Indian Journal of Pharmacology. 29(3): 162-167.

Carrol, P.B., Moura, A.S., Rojas, E., and Atwater, I. 1994. The diabetogenic agent alloxan increases K+ permeability by a mechanism involving ATP-sensitive K+ channels in mouse pancreatic beta cells. Molecular and Cellular Biochemistry. 140(12): 127-136.

Chattopadhyay, R.R. 1999. Possible mechanism of antihyperglycemic effect of Azadirachta indica leaf extract: part V. Journal of Ethnopharmacology. 67(3):373-376.

Cheong, S.H., Kim, M.Y., Sok, D.E., Hwang, S.Y., Kim, J.H., Kim, H.R., Lee, J.H., Kim, Y.B., and Kim, M.R. 2010. Spirulina Prevents Atherosclerosis by Reducing Hypercholesterolemia in Rabbits Fed a High-Cholesterol Diet. Journal of Nutritional Science and Vitaminology. 56: 34-40.

Friedewald, W.T., Levy, R.I., and Fredrickson, D.S. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 18(6): 499-02.

Halim, E.M. 2003. Lowering of blood sugar by water extract of Azadirachta indica and Abroma augusta in diabetes rats. Indian Journal of Experimental Biology. 41(6): 636-640.

Iwata, K., Inayama, T., and Kato, T. 1990. Effects of Spirulina platensis on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats. Journal of Nutritional Science and Vitaminology. 36:165-71.

Itemobong, S.E., Item, J.A., Henry, D.A., Itoro, F.U., Oboso, E.E., and Patrick, E.E. 2010. Effects of ethanol extract of Azadirachta indica leaves on some immunological and haematological parameters of diabetic Wistar rats. African Journal of Pharmacy and Pharmacology. 4(3): 104-108.

Jelodar, G.A., Maleki, M., Motadayen, M.H., and Sirus, S. 2005. Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. Indian Journal of Medical Sciences. 59: 64-69.

Khosla, P., Bhanwra, S., Singh, J., Seth, S., and Srivastava, R.K. 2000:.A study of hypoglycaemic effects of Azadirachta indica (Neem) in normal and alloxan diabetic rabbits. Indian Journal of Physiology and Pharmacology. 44(1):69-74.

Lamberg, S.L., and Rothstein, R. 1977. Laboratory manual of Hematology and urinalysis. Avi Publishing Company, Inc. Wesport Connecticent, U.S.A.

Layam, A., and Reddy, C.L.K. 2006. Antidiabetic Property of Spirulina. Diabetologia Croatica. 35(2): 29-33.

Maged, M.Y., Rahiem, A., and Nehad, E.R. 2004. Alterations in body weight, protein profile, nonprotein nitrogen constituents and kidney structure in diabetic rats under glibenclamide treatment. Journal of the Islamic University of Gaza, (Natural Sciences Series). 12(1): 37-54.

Mani, U., Desai, S., and Iyer, U. 2000. Studies on the long-term effect of spirulina supplementation on serum lipid profile and glycated proteins in NIDDM patients. Journal of nutraceuticals, functional & medical foods. 2(3): 25-32.

Melmed, S., Polonsky, K.S., Larsen, P.R., and Kronenberg, H.M. 2012. Williams textbook of endocrinology (12[th]ed.). Philadelphia: Elsevier/Saunders. Pp. 1371–1435.

Mendez, J.D., and Ramos, H.G. 1994. Animal models in diabetes research. Archives of Medical Research. 25(4): 367-375.

Moura, L.P., Puga, G.M., Beck, W.R., Teixeira, I.P., Ghezzi, A.C., Silva, G.A., and Mello, M.R. 2011. Exercise and spirulina control non-alcoholic hepatic steatosis and lipid profile in diabetic Wistar rats. Lipids in Health and Disease.10:77.

Pandey, J.P., Tiwari, A., Mishra, G., and Mishra, R.M. 2011. Role of Spirulina maxima in the Control of Blood Glucose Levels and Body Weight in Streptozotocin induced Diabetic Male Wistar rats. Journal of Algal Biomass Utilization. 32 (4): 35–37.

Rodríguez-Hernández, A., Blé-Castillo, J.L., Juárez-Oropeza, M.A., Díaz-Zagoya, J.C. 2001. Spirulina maxima prevents fatty liver formation in CD-1 male and female mice with experimental diabetes. Life Sciences. 69(9): 1029-1037.

Soto, C., Muriel, P., and Reyes, J.L. 1994. Pancreatic lipid peroxidation in alloxan-induced diabetes mellitus. Archives of Medical Research. 25(4): 377-380.

Soto, C., Recoba, R., Barrón, H., Alvarez, C., and Favari, L. 2003. Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 136(3): 205–212.

Trinder, P. 1969. Cholesterol determination test. Clinical Biochemistry. 6: 24-25.

Usman, L.A., Ameen, O.M., Ibiyemi, S.A., and Muhammad, N.O. 2005. The extraction of proteins from the neem seed (Indica azadirachta A. Juss). African Journal of Biotechnology. 4: 1142-1144.

Van der Nat, J.M., van der Sluis, W.G., deHaan, A.H., de Silva, K.T., and Labadie, R.P. 1986. Ethnopharmacological study of Azadirachta indica. A conceptual evaluation. Planta Medica. 52: 552-560.

Wild, S., Roglic, G., Green, A., Sicree, R., and King, H. 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care. 27 (5): 1047–1053.