

VIGNA UNGUICULATA LINN. WALP. SEED OIL EXHIBITING ANTIDIABETIC EFFECTS IN ALLOXAN INDUCED DIABETIC RATS

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Antidiabetic effects of Vigna unguiculata Linn. Walp (barbati) seed oil was investigated in albino rats by administration of single dose of alloxan monohydrate [110 mg/kg body weight (b.wt)]. The seed oil of barbati at doses of 100 and 200 mg/kg.b.wt was administered as single dose per day to diabetes-induced rats for a period of 21 days. The effect of oil on blood glucose level was measured in the diabetic rats. Serum lipid profile [total cholesterol (TC), triglycerides (TGs), low density (LDL) and high density lipoprotein (HDL)] and enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were also determined. The activities were also compared to that produced by a standard antidiabetic agent, glipizide. Levels of blood glucose, TC, TGs, LDL, ALT, AST and ALP decreased and HDL increased in alloxan induced diabetic rats after treatment with 200 mg/kg.b.wt barbati seed oil for 21 days. The present study reported that seed oil of barbati may be very useful for the improvement of the complications of diabetes.

Keywords: Alloxan, Glipizide, *Vigna unguiculata* Linn., Hyperglycemic, Seed oil

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder which involves disturbances in the metabolism of carbohydrate, fat and protein, and characterised by hyperglycemia (Fuentes *et al.* 2005), abnormalities of lipoprotein (Scoppola *et al.* 2001), defect in reactive oxygen specific scavenging enzymes (Bolajoko *et al.* 2008), altered intermediary metabolism of major food substances (Unwin, Sobngwi and Alberti 2001) and raised basal metabolic rate (Avesani *et al.* 2001). There are several drugs for the treatment of diabetes but they have prominent side effects. There has been increasing demand for the use of plant products with antidiabetic activity.

Vigna unguiculata Linn. Walp. (Bengali name: *barbati*, English name: cowpea), is an annual, warm season herbaceous legume (Davis *et al.* 1991). It was reported to have originated in Asia, Africa and South America (Ng and Marechal 1985). It is used as medicine to treat stubborn boils by mixing the seed powder with oil (Duke 1990). The seed

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is used to strengthen the stomach and is also diuretic. Boiled seeds are eaten to destroy worms in the stomach (Chopra, Nayar and Chopra 1986). To treat amenorrhea, infusion of seed can be taken orally whilst powdered roots eaten with porridge is used to treat chest pain, epilepsy and painful menstruation (Van Wyk and Gericke 2000). Cooked seeds and roots of other herbs are used orally to treat blood in urine and bilharzias (Kritzinger, Lall and Aveling 2005). Zia-ul-Haq *et al.* (2010) reported that barbati seed oil contains tocopherols. Tocopherols are the most important lipophilic antioxidants and are believed to play a preventive role in diseases associated with oxidative stress like central neurodegenerative diseases, age-related muscular degeneration, cancer, cataracts, cardiovascular diseases and diabetes mellitus (Brigelius-Flohe *et al.* 2002). Duyff (2006) and Pollan (2008) reported that kidney beans, navy beans and green beans are good sources of omega 3 fatty acids (alpha linolenic acid, ALA). It is also reported that omega-3 fatty acids improve blood sugar levels in those with diabetes and lowers cholesterol levels (Duyff 2006).

Some workers have studied on antidiabetic effect of seed extract from *Syzygium cumini* seeds (Farswan *et al.* 2009), *Prunus amygdalus* seeds (Teotia and Singh 1997), *Cassia auriculata* seeds (Jalalpure *et al.* 2004), *Ocimum sanctum* Linn. seed (Gupta *et al.* 2006), sesame oil (Sankar *et al.* 2011) and *Nigella sativa* seeds (Al-Hader, Aqel and Hasan 1993).

However, no such scientific data are available regarding the effect of local *barbati* seed (LBS-2) oil on blood glucose level and its effect on lipid profile as well as some clinically important enzymes. The present study was designed to investigate these effects at different concentrations of oil with test animals.

METHODS

Animal and Diet

Fourty rats with body weight (b.wt) of 111–170 g, were bought from the International Centre for Diarrhoeal Disease Research, Bangladesh for the present study. The rats were housed in standard plastic cages at room temperature (28°C to 30°C) and relative humidity of 50% to 55% for 4 to 6 days prior to the experimental work. During the experimental period, standard pellet diets were fed to the rats and water was supplied *ad libitum*. This study was done according to the guideline of the Institutional Animal Ethics Committee. This study was approved by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) of the University of Rajshahi.

Collection of Seeds

Ripe pods of *barbati* used in this work were collected in the year 2007 from the experimental plot located at Rajshahi City, Rajshahi, Bangladesh. The authenticity of the *barbati* was identified by Professor A. T. M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh. The seeds were separated manually from the flesh of the fruits and washed several times with water to remove foreign materials. Afterward, the seeds were dried in the sunlight for 4 consecutive days and again in an electric oven at 40°C until a constant weight were reached. The seeds were ground to a fine powder, packed and stored in a refrigerator at 4°C prior to analysis.

Extraction of Oil from Seed

For solvent extraction (Soxhlet method), 500 g of ground seeds were placed into a cellulose paper cone and extracted using n-hexane in a 5-l Soxhlet extractor for 8 h (Pena, Anguiano and Arredondo 1992). By using rotary evaporator the oil was recovered and residual solvent was removed by drying in an oven at 60°C for 1 h.

Induction of Diabetics

Diabetes mellitus was induced once by a single intraperitoneal injection dose of freshly prepared alloxan with normal saline (110 mg/kg.b.wt). Rats were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Diabetics were developed and stabilised in these alloxan treated rats over a period of 14 days (Szkudelski, Kandulska and Okulicz 1998; Akhtar *et al.* 2007). After 14 days of alloxan administration, plasma glucose levels of each rat were determined. Rats with a fasting blood glucose level greater than 250 mg/dL were considered as diabetic and included in the present study.

Preparation of Solution for Oral Administration

Suspension of oral hypoglycemic drug (glipizide) (0.6 mg/kg.b.wt): About 6 mg of glipizide (trade name Diactin, contains glipizide BP 5 mg/tablet; Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh) was suspended uniformly in 5 mL distilled water and mixed well with a vortex mixture. This suspension was fed orally to the experimental rats at a dose of 0.6 mg/kg.b.wt (Kamal 2006).

Grouping of the Rats

Weights of individual rats were measured and the rats were grouped as shown in Table 1. Each group contained eight rats: Group A - "non diabetic", fed normal diet; Group B - alloxan-treated diabetic control, fed normal diet; Group C - alloxan-treated diabetic rats treated with oral hypoglycemic drug glipizide solution (0.6 mg/kg.b.wt), fed normal diet; Group D and Group E - alloxan-induced diabetic rats, fed LBS-2 oil at a dose of 100 mg/kg.b.wt and 200 mg/kg.b.wt, respectively, along with normal diet per day using a feeding needle.

Table 1: Grouping of experimental rats.

| Group | No. of rats | Average body weight (g) | Age (week) | Daily dose (mg/kg.b.wt) |
|-------|-------------|-------------------------|------------|----------------------------|
| A | 8 | 111.80±5.6 | 4-7 | nondiabetic |
| B | 8 | 140.50±5.2 | 4-7 | diabetic control |
| C | 8 | 153.90±4.7 | 4-7 | diabetic + glipizide (0.6) |
| D | 8 | 155.70±3.4 | 4-7 | diabetic + LBS-2 oil (100) |
| E | 8 | 170.40±5.7 | 4-7 | diabetic + LBS-2 oil (200) |

Collection and Treatment of Samples

Blood of the rats were collected from their tail (by cutting the edge of tails) at the 1st, 7th and 14th day of the experiment. After 21 days of treatment, rats were sacrificed and their blood was collected for the estimation of blood parameters. After every week of oral administration, rats were kept fasting overnight, and the fasting blood was collected. Serum was obtained immediately by centrifugation (15 min at 4000 rpm), which was used for the measurement of various biochemical parameters. All analyses were carried out within 24 h of blood collection.

Analysis

Serum glucose level was estimated by glucose oxidase phenol aminophenazone (GOD-PAP) method using commercial kit (Human, Germany) with the help of UV-visible spectrophotometer (Shimadzu, Japan) at 500 nm (Teuscher and Richterich 1971; Barham and Trinder 1972).

Serum total cholesterol (TC) level was estimated by cholesterol oxidase phenol aminophenazone (CHOD-PAP) method by using commercial kit (Human, Germany) with the help of UV-visible spectrophotometer at 500 nm (Naito and Kaplan 1984).

Serum triglyceride (TG) level was estimated by GPO-PAP method using commercial kit (Human, Germany) with the help of UV-visible spectrophotometer at 500 nm (Jacobs and Vandemark 1960).

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample precipitate with phosphotungstate and magnesium ions. The supernatant contains high density lipoproteins (HDL). The HDL cholesterol is spectrophotometrically measured at 500 nm (Grove 1979).

Aspartate aminotransferase (AST) in serum was estimated by AST assay kit (Human, Germany) using spectrophotometer at 340 nm (Wallnofer, Schmidt and Schmidt 1974; Bergmeyer, Horder and Rej 1986a). Alanine aminotransferase (ALT) in serum was estimated by ALT assay kit (Human, Germany) using spectrophotometer at 340 nm (Bergmeyer, Horder and Rej 1986b). Serum alkaline phosphatase (ALP) activity was determined by using the commercially available kits according to the method of King and King (1954).

Statistical Analysis

The results are expressed as the mean \pm standard deviation (S.D.) of triplicate analyses. All statistical comparisons were performed using a one-way analysis of variance (ANOVA) followed by a multiple two-tailed t-test. Differences were considered significant at a *p* level of 0.05 or lower.

RESULTS

The results obtained with untreated diabetic rats (diabetic control Group B) and diabetic rats treated with LBS-2 oil at the dose of 100 mg/kg.b.wt and 200 mg/kg.b.wt respectively on serum glucose, lipid profiles, enzymes and b.wt were compared with non diabetic healthy controls and glipizide was used as reference drug. In diabetic rats there was a

significant decrease of b.wt as compared to non diabetic rats (Fig. 1). After 21 days of LBS-2 oil administration, the b.wt increased. It was seen that the treatment with LBS-2 oil at a dose of 100 mg/kg.b.wt increased the b.wt from 155.70 to 163.23 g and at 200 mg/kg.b.wt increased the b.wt from 170.40 to 177.31 g, while at the diabetic control Group B the b.wt decreased from 140.50 to 123.21 g.

Alloxan-induced diabetes resulted in a significant elevation in blood glucose level in comparison to the diabetic control rats (Table 2). After the administration of LBS-2 oil to diabetic animals for 21 days, a significant reduction in blood glucose level was noted and at the dose of 200 mg/kg.b.wt serum glucose level was found to be very close to the non diabetic control Group A.

The serum TC and TG increased significantly in diabetic rats as compared to non diabetic rats (Table 3). After 21 days of consumption of LBS-2 oil at a dose of 200 mg/kg.b.wt brought the levels of these blood lipids to near normal values. Serum HDL level decreased whereas LDL level increased significantly in diabetic rats but after treatment with the fruit extract, HDL level increased whereas LDL level decreased significantly. It was seen that, at the administration of a dose of 200 mg/kg.b.wt the lipid profile was near to the values of non diabetic control rats.

Table 2: Effect of LBS-2 oil on serum glucose level in experimental rats.

| Groups | Serum glucose level (mg/dl) | | | |
|-------------------------------------------------|-----------------------------|---------------------------|---------------------------|---------------------------|
| | 1st day | 7th day | 14th day | 21st day |
| Non diabetic (Group A) | 110±0.92 | 112±0.88 | 118±0.93 | 121±0.97 |
| Diabetic control (Group B) | 279±0.85 [†] | 297.25±0.85 [†] | 312.22±0.93 [†] | 333.07±0.80 [†] |
| Diabetic + glipizide (0.6 mg/kg.b.wt) (Group C) | 287.3±9.8 | 189.35±0.50 ^{**} | 129.17±0.88 ^{**} | 109.75±1.62 ^{**} |
| Diabetic + LBS-2 oil (100 mg/kg.b.wt) (Group D) | 285.2±3.2 | 250±0.53 | 197.15±0.86 ^{**} | 147.16±0.53 ^{**} |
| Diabetic + LBS-2 oil (200 mg/kg.b.wt) (Group E) | 281.8±0.86 | 239.15±0.58 | 173.05±0.82 ^{**} | 123.9±1.94 ^{**} |

Notes: ^{**} $p < 0.01$ and ^{*} $p < 0.05$ compared to diabetic control Group B. [†] $p < 0.01$ compared to non diabetic Group A.

The serum enzymes (AST, ALT and ALP) level of diabetic rats also increased significantly as compared to non diabetic control rats (Table 4). After 21 days of LBS-2 oil administration, the serum enzymes (AST, ALT and ALP) levels of diabetic rats at a dose of 200 mg/kg.b.wt significantly reduced as compared to diabetic control rats.

Table 3: Effects of LBS-2 oil on serum TC, TG, HDL and LDL cholesterol level in experimental rats.

| Groups | Cholesterol (mg/dl) | TG (mg/dl) | HDL cholesterol (mg/dl) | LDL cholesterol (mg/dl) |
|-------------------------------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| Non diabetic (Group A) | 65±3.22 | 74±5.70 | 45±1.88 | 100.0±10.3 |
| Diabetic control (Group B) | 162±0.83 [†] | 120.25±5.70 [†] | 21.6±0.88 [†] | 140.2±10.3 [†] |
| Diabetic + glipizide (0.6 mg/kg.b.wt) (Group C) | 68±2.16 ^{**} | 63.75±2.12 ^{**} | 42.9±1.42 [*] | 108±5.6 [*] |
| Diabetic + LBS-2 oil (100 mg/kg.b.wt) (Group D) | 96±0.81 ^{**} | 65.25±0.95 ^{**} | 37.03±0.95 ^{**} | 120±6.2 ^{**} |
| Diabetic + LBS-2 oil (200 mg/kg.b.wt) (Group E) | 87.75±3.30 ^{**} | 62.0±1.29 ^{**} | 40.9±1.31 [*] | 117.5±4.5 ^{**} |

Notes: ^{**} $p < 0.01$ and ^{*} $p < 0.05$ compared to diabetic control Group B. [†] $p < 0.01$ compared to non diabetic Group A.

Table 4: Effects of LBS-2 oil on ALT, AST and ALP levels in experimental rats.

| Biochemical parameter | ALT (U/L) | AST (U/L) | ALP (U/L) |
|-------------------------------------------------|-------------------------|------------------------|-------------------------|
| Non diabetic (Group A) | 44.95±1.22 | 77±1.20 | 146±0.62 |
| Diabetic control (Group B) | 63.25±1.7 [†] | 123±1.10 [†] | 177.75±2.2 [†] |
| Diabetic + glipizide (0.6 mg/kg.b.wt) (Group C) | 37.75±1.4 ^{**} | 101±2.16 ^{**} | 133±0.82 ^{**} |
| Diabetic + LBS-2 oil (100 mg/kg.b.wt) (Group D) | 51±2.36 ^{**} | 116±1.65 ^{**} | 141±2.08 ^{**} |
| Diabetic + LBS-2 oil (200 mg/kg.b.wt) (Group E) | 48.093 ^{**} | 108±2.64 ^{**} | 128±2.5 ^{**} |

Notes: ^{**} $p < 0.01$ and ^{*} $p < 0.05$ compared to diabetic control Group B. [†] $p < 0.01$ compared to non diabetic Group A.

DISCUSSION

In the present study, the results showed that oral administration of *barbati* seed oil significantly decreases the fasting blood glucose level in the diabetic induced rats as compared to controls. This reduction might be due to the presence of tocopherol in the *barbati* seed oil. However, the reduction in the blood glucose level is less than that brought about by the standard drug, glipizide. Sankar *et al.* (2011) reported that sesame seed oil reduces the glucose level of type 2 diabetic patient. Hyperglycemia is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to

heart kidney, eyes, nerves, liver, small and large vessels and gastro intestinal system (Tunali and Yanardag 2006).

Table 5: Effects of LBS-2 oil on body weight in experimental rats.

| Group | Body weight (g) | | | |
|-------------------------------------------------|-------------------------|---------------------------|---------------------------|--------------------------|
| | 1st day | 7th day | 14th day | 21st day |
| Non-diabetic (Group A) | 111.80±5.6 | 115.90±3.12 | 122.51±4.16 | 130.31±5.1 |
| Diabetic control (Group B) | 140.50±5.2 [†] | 135.44±8.11 [†] | 130.6±5.11 [†] | 123.11±3.1 [†] |
| Diabetic + glipizide (0.6 mg/kg.b.wt) (Group C) | 150.90±4.7 | 155.15±5.22 ^{**} | 157.02±5.41 ^{**} | 158.55±3.3 [*] |
| Diabetic + LBS-2 oil (100 mg/kg.b.wt) (Group D) | 155.70±3.4 | 158.91±4.5 ^{**} | 160.76±5.67 ^{**} | 163.13±8.2 ^{**} |
| Diabetic + LBS-2 oil (200 mg/kg.b.wt) (Group E) | 170.40±5.7 | 172.12±4.12 ^{**} | 175.3±6.3 ^{**} | 177.31±8.2 [*] |

Notes: ^{**} $p < 0.01$ and ^{*} $p < 0.05$ compared to diabetic control Group B. [†] $p < 0.01$ compared to non-diabetic Group A.

Lipids play a vital role in the pathogenesis of diabetes mellitus. In diabetes, the increase in blood glucose levels is usually accompanied by an increase in plasma cholesterol, TGs, LDL and decreases in HDL (Mitra *et al.* 1995). The increased levels of serum lipids in diabetes represent a risk factor for coronary heart disease (Al-Shamaony, Al-Khazraji and Twaij 1994). Under normal circumstances, insulin activates lipoprotein lipase and hydrolyzes TGs. Insulin increases uptake of fatty acids into adipose tissue and increases TGs synthesis. Moreover, insulin inhibits lipolysis. In insulin-deficient diabetes, the concentration of serum free fatty acids is elevated, as a result of free fatty acid outflow from fat depots, where the balance of the free fatty acids esterification-triglyceride lipolysis cycle is displaced in favour of lipolysis (Shirwaikar *et al.* 2004). Thus an excess fatty acid in the plasma produced by the alloxan-induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances, along with excess TGs formed in the liver may be discharged into the blood in the form of lipoproteins (Bopama *et al.* 1997). HDL is an antiatherogenic lipoprotein. It transports cholesterol from peripheral tissues into the liver and thereby acts as a protection factor against coronary heart disease. Oral administration of *barbati* seed oil lowers serum lipids and also increases the serum HDL-cholesterol level in diabetic rats so might be considered as a substitute of drugs to combat diabetic associated complications.

The liver is an important insulin-dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes (Seifter and England 1982). The increase in the activities of serum glutamic pyruvic transaminase (SGPT) (ALT), serum glutamic oxaloacetic transaminase (SGOT) (AST) and ALP indicated that diabetes might be induced due to liver dysfunction (Ohaeri 2001). Therefore, increase in the activities of SGPT, SGOT and ALP, may be mainly due to the leakage of these enzymes from the cytosol of hepatic cells into the blood stream (Navarro *et al.* 1993). On

the contrary in our present study the treatment of the diabetic rats with *barbati* seed oil caused reduction in the activity of these enzymes in serum compared to the mean values of diabetic group and consequently may alleviate liver damage caused by alloxan-induced diabetes.

CONCLUSION

Our finding shows that oral administration of *barbati* seed oil produces significant hypoglycemic and hypolipidemic effect which lowers glucose level as well as TC and TG, and at the same time increases HDL-cholesterol to near normal range in alloxan-induced diabetic rats. This investigation reveals that *barbati* seed oil has potent antidiabetic and hypolipidemic effects in alloxan-induced diabetic rats.

REFERENCES

- AKHTAR, M. A., RASHID, M., WAHED, M. I. I., ISLAM, M. R., SHAHEEN, S. M., ISLAM, M. A. *et al.* (2007) Comparison of long-term antihyperglycemic and hypolipidemic effects between *Coccinia cordifolia* (Linn.) and *Catharanthus roseus* (Linn.) in alloxan-induced diabetic rats, *Research Journal of Medicine and Medical Sciences*, 2: 29-34.
- AL-HADER, A., AQEL, M. & HASAN, Z. (1993) Hypoglycemic effects of the volatile oil of *Nigella sativa* seeds, *Pharmaceutical Biology*, 31: 96-100.
- AL-SHAMAONY, L., AL-KHAZRAJI, S. M. & TWAJJ, H. A. (1994) Hypoglycaemic effect of *Artemisia herba alba*, II, effect of a valuable extract on some blood parameters in diabetic animals, *Journal of Ethnopharmacology*, 43: 167-171.
- AVESANI, C. M., CUPPARI, L., SILVA, A. C., SIGULEM, D. M., CENDOROGLO, M., SESSO, R. *et al.* (2001) Resting energy expenditure in pre-dialysis diabetic patients, *Nephrology, Dialysis, Transplantation*, 16: 556-565.
- BARHAM, D. & TRINDER, P. (1972) An improved colour reagent for the determination of blood glucose by oxidase system, *The Analyst*, 97: 142-145.
- BERGMEYER, H. U., HORDER, M. & REJ, R. (1986a) International Federation of Clinical Chemistry (IFCC) Scientific Committee, analytical section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes, part 2, IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1), *Journal of Clinical Chemistry & Clinical Biochemistry*, 24: 497-510.
- BERGMEYER, H. U., HORDER, M. & REJ, R. (1986b) International Federation of Clinical Chemistry (IFCC) Scientific Committee, analytical section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes, part 3, IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2), *Journal of Clinical Chemistry & Clinical Biochemistry*, 24: 481-495.
- BOLAJOKO, E. B., MOSSANDA, K. S., ADENIYI, F., AKINOSUN, O., FASANMADE, A. & MOROPANE, M. (2008) Antioxidant and oxidative stress status in type 2 diabetes and diabetic foot ulcer, *South African Medical Journal*, 98: 614- 617.

- BOPAMA, K. N., KANNA, J., SUSHMA, G., BALARAMAN, R. & RATHOD, S. P. (1997) Antidiabetic and antihyperlipidemic effects of neem seed kernel powder on alloxan diabetic rabbits, *Indian Journal of Pharmacology*, 29: 162-167.
- BRIGELIUS-FLOHE, R., KELLY, F. J., SALONEN, J. T., NEUZIL, J., ZINGG, J. M. & AZZI, A. (2002) The European perspective on vitamin E: Current knowledge and future research, *The American Journal of Clinical Nutrition*, 76: 703-716.
- CHOPRA, R. N., NAYAR, S. L. & CHOPRA, I. C. (1986) *Glossary of Indian medicinal plant (including the supplement)* (New Delhi: Council of Scientific and Industrial Research).
- DAVIS, D. W., OELKE, E. A., OPLINGER, E. S., DOLL, J. D., HANSON, C. V. & PUTNAM, D. H. (1991) *Cowpea, in alternative field crops manual* (USA: Cooperative Extension, University of Wisconsin-Extension and Centre for Alternative Plant and Animal Products, and the Minnesota Extension Service, University of Minnesota).
- DUKE, J. A. (1990) Introduction to food legumes, IN: S. R. Singh (Ed.). *Insect pests of tropical food legumes*, pp. 1-42 (Chichester, United Kingdom: John Wiley & Sons).
- DUYFF, R. L. (2006), *Complete food and nutrition guide*, 3rd edition (Hoboken, NJ: American Dietetic Association and John Wiley & Sons).
- FARSWAN, M., MAZUMDER, P., PARCHA, V. & UPAGANLAWAR, A. (2009) Modulatory effect of *Syzygium cumini* seeds and its isolated compound on biochemical parameters in diabetic rats, *Pharmacognosy Magazine*, 5: 127-133.
- FUENTES, N. L., SAGUA, H., MORALES, G., BORQUEZ, J., MARTIN, S. A., SOTO, J. *et al.* (2005) Experimental antihyperglycemic effect of diterpenoids of llareta *Azorella compacta* (Umbelliferae) phil in rats, *Phytotherapy Research*, 19: 713-716.
- GROVE, T. H. (1979) Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium, *Clinical Chemistry*, 25: 560-564.
- GUPTA, S., MEDIRATTA, P. K., SINGH, S., SHARMA, K. K. & SHUKLA, R. (2006) Antidiabetic, antihypercholesterolaemic and antioxidant effect of *Ocimum sanctum* (Linn.) seed oil, *Indian Journal of Experimental Biology*, 44: 300-304.
- JACOBS, N. J. & VANDEMARK, P. J. (1960) The purification and properties of the alpha-glycerophosphate-oxidizing enzyme of *Streptococcus faecalis* 10C1, *Archives of Biochemistry and Biophysics*, 88: 250-255.
- JALALPURE, S. S., PATIL, M. B., PAI, A., SHAH, B. N. & SALAHUDDIN, M. D. (2004) Antidiabetic activity of *Cassia auriculata* seeds in alloxan induced diabetic rats, *Nigerian Journal of Natural Products and Medicine*, 8: 22-23.
- KAMAL, D. M. M. (2006) *Drug directory*, 6th edition, pp 121-125.
- KING, P. R. N. & KING, E. J. (1954) Estimation of plasma phosphate by determination of hydrolysed phenol with amino-antipyrine, *Journal of Clinical Pathology*, 7: 322-326.
- KRITZINGER, Q., LALL, N. & AVELING, T. A. S. (2005) Antimicrobial activity of cowpea (*Vigna unguiculata*) leaf extracts, *South African Journal of Botany*, 71: 45-48.

- MITRA, S. K., GOPUMADHAVAN, S., MURALIDHAR, T. S., ANTURLIKAR, S. D. & SUJATHA, M. B. (1995) Effect of D-400, a herbomineral preparation on lipid profile, glycated haemoglobin and glucose tolerance in streptozotocin induced diabetes in rats, *Indian Journal of Experimental Biology*, 33: 798-800.
- NAITO, H. K. & KAPLAN, A. (1984) *Clinical chemistry*, pp. 437, 1194-1206 (St. Louis, MO: C. V. Mosby Co.).
- NAVARRO, C. M., MONTILLA, P. M., MARTIN, A., JIMENEZ, J. & UTRILLA, P. M. (1993) Free radicals scavenger and antihepatotoxic activity of rosmarinus, *Planta Medica*, 59: 312-314.
- NG, N. Q. & MARECHAL, R. (1985) Cowpea taxonomy, origin and germplasm, IN: R. S. Singh & K. O. Rachie (Eds.). *Cowpea research, production and utilization*, pp. 11-21 (New York: John Wiley and Sons).
- OHAERI, O. C. (2001) Effect of garlic oil on the levels of various enzyme in the serum and tissue of streptozotocin diabetic rats, *Bioscience Reports*, 21: 19-24.
- PENA, D. G., ANGUIANO, R. G. L. & ARREDONDO, J. J. M. (1992) Modification of the method 1 AOAC (CB-method) for the detection of aflatoxins, *Bulletin of Environmental Contamination and Toxicology*, 49: 485-489.
- POLLAN, M. (2008) *In defense of food: An eater's manifesto* (New York: Penguin Press).
- SANKAR, D., ALI, A., SAMBANDAM, G. & RAO, R. (2011) Sesame oil exhibits synergistic effect with anti-diabetic medication in patients with type 2 diabetes mellitus, *Clinical Nutrition*, 30: 351-358.
- SCOPPOLA, A., MONTECCHI, F. R., MENZINGER, G. & LALA, A. (2001) Urinary mevalonate excretion rate in type 2 diabetes: Role of metabolic control, *Atherosclerosis*, 156: 357-361.
- SEIFTER, S. & ENGLAND, S. (1982) Energy metabolism, IN: I. Arias, H. Popper & D. Schacter (Eds.). *The Liver: Biology and pathology*, pp. 219-249 (New York: Raven Press).
- SHIRWAIKAR, A., RAJENDRAN, K., KUMAR, C. D. & BODLA, R. (2004) Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin-nicotinamide type 2 diabetic rats, *Journal of Ethnopharmacology*, 91: 171-175.
- SZKUDELSKI, T., KANDULSKA, K. & OKULICZ, M. (1998) Alloxan in vivo does not only exert deleterious effects on pancreatic β cells, *Physiology Research*, 47: 343-346.
- TEOTIA, S. & SINGH, M. (1997) Hypoglycemic effect of *Prunus amygdalus* seeds in albino rabbits, *Indian Journal of Experimental Biology*, 35: 295-296.
- TEUSCHER, A. & RICHTERICH, P. (1971) Enzymatic method of glucose, *Schweizerische Medizinische Wochenschrift*, 101: 345-390.
- TUNALI, S. & YANARDAG, R. (2006) Effect of vanadyl sulfate on the status of lipid parameters and on stomach and spleen tissues of streptozotocin-induced diabetic rats, *Pharmacological Research*, 53: 271-277.
- UNWIN, N., SOBNGWI, E. & ALBERTI, K. G. M. M. (2001) Type 2 diabetes: The challenge of preventing a global epidemic, *Diabetes International*, 11: 4-8.

VAN WYK, B. E. & GERICKE, N. (2000) *People's plants: A guide to useful plants of Southern Africa*, pp. 192 (Pretoria, South Africa: Briza Publications).

WALLNOFFER, H., SCHMIDT, E. & SCHMIDT, F. W. (1974) *Synopsis der Leberkrankheiten* (Stuttgart: Georg Thieme Verlag).

ZIA-UL-HAQ, M., AHMAD, S., CHIAVARO, E., MEHJABEEN & AHMED, S. (2010) Studies of oil from cowpea [*Vigna unguiculata* (L.) Walp] cultivars commonly grown in Pakistan, *Pakistan Journal of Botany*, 42: 1333-1341.