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Antidiabetic and hypolipidaemic effects of few common plants extract in Type 2 diabetic patients at Bengal

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Abstract
Medicinal plants are used in rural India to treat diabetes due to their traditional acceptability and availability. The plants are used as a crude extracts or as mixtures to treat diabetes. The objective of this study was to investigate the effects of aqueous extracts of *Acalypha indica*, *Allium sativum*, *Allium cepa*, *Azadirachta indica*, *Musa sapientum*, *Mangifera indica*, *Murraya koenigii*, *Ocimum sanctum*, *Phyllanthus amarus* and *Tinospora cordifolia* on blood glucose and lipid profile in Type 2 diabetic patients. Four hundred Type 2 diabetics were selected randomly from 828 patients. Ten experimental and ten control groups were formed each group comprising 20 patients. Aqueous extract of a particular plant was given to a particular experimental group for two months maintaining homogeneity in other variables to allow statistical analysis. Blood samples were collected at monthly intervals and biochemical parameters were analyzed. Fasting blood sugar level was lowered by *Mangifera indica* (136±14 to 130±12 mg/dl, $p<0.02$), *Murraya koenigii* (134±9 to 129±10 mg/dl, $p<0.03$) and *Azadirachta indica* (125±12 to 120±9 mg/dl, $p<0.03$). *Ocimum sanctum*s not only lower total cholesterol (TC) (142±14 to 137±15 mg/dl, $p<0.03$) and LDL (91±14 to 85±19 mg/dl, $p<0.03$) level but also increase HDL (25±3 to 27±4 mg/dl, $p<0.03$) level. In addition, *Allium cepa*, *Mangifera indica*, *Murraya koenigii* and *Phyllathus amarus* showed significant ($p<0.03$) reduction in triglycerides (TG), TC, and very low density lipoproteins (VLDL) levels. *Mangifera indica*, *Murraya koenigii*, *Ocimum santum*, *Phyllanthus amarus*, *Allium cep* and *Azadirachta indica* exhibited anti-diabetic as well as hypolipidemic effects in Type 2 diabetic patients.

Key words: *Plant extracts; Biochemical parameters; Anti-diabetic, Hypolipidemic*

Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetes is divided into two etiopathogenic categories. In the first category, Type 1 diabetes occurs due to absolute deficiency of insulin and 5-10% of people are affected by this type of diabetes. In the other, much more prevalent category, Type 2 diabetes occurs mostly due to a combination of insulin resistance and an inadequate compensatory insulin secretory response.[1] Type 2 diabetes is on the rise not only in rural India but also in rural Bengal.[2] Type 2 diabetes may be complicated by hypertension, poor metabolic control, smoking, obesity and hyperlipidemia.[3] Modern medicines like biguanides, sulphonylureas and thiazolidinediones are available for the treatment of Type 2 diabetes but they have undesired effects.[4] Medicinal plants have been always an exemplary source of drugs. The ethno-botanical information reports about 800 plants possessing anti-diabetic potentials.[5] Many medicinal plants and their products (natural principles and crude extracts) have been either mentioned or used in the Indian traditional system of medicine and have been shown to possess anti-diabetic activities.[6,7] Medicinal plants have been investigated with respect to suppression of metabolic conversion of carbohydrates to glucose in the gut and subsequent absorption from the intestine.[8] The importance of medicinal plants has received a lot of attention for the management of Type 2 Diabetes in the Indian subcontinent due to various infrastructural gaps in the health care system including its socio-cultural, socio-economic, socio-political and ethno-demographic/anthropological domains. Therefore, alternative approaches like herbal remedies are increasing.[9]

In the present study, depending on its wide availability in the IIT Kharagpur Campus, medicinal plants such as *Acalypha indica*, *Allium sativum*, *Allium cepa*, *Azadirachta*, *Musa sapientum*, *Mangifera indica*, *Murraya koenigii*, *Ocimum sanctum*, *Phyllanthus amarus* and *Tinospora cordifolia* were selected to investigate their anti diabetic and hypolipidemic potentials in Type 2 diabetic patients.

Materials and Methods

The study was conducted at BC Roy Technology Hospital and School of Medical Science and Technology, IIT Kharagpur, West Bengal, India. The following criteria were used to include or exclude the patients in the study.[10]

Inclusion Criteria:

- Type 2 diabetic patients with fasting plasma glucose (FPG) level equal to or greater than 140 mg/dl of blood (WHO Study Group on diabetes mellitus, 1985, Geneva

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technical report series 727) (WHO, 1985) without any detectable/visible complications.

- ❖ Mild Type 2 diabetic patients (FPG) < 180 mg/dl) without oral hypoglycemic, anti- hypertensive and hypolipidemic drugs and drugs acting on the hepato-billiary and renal systems.
- ❖ Onset of diabetes within 5 years and with no family history of diabetes mellitus/gestational diabetes.
- ❖ The patients and control subjects were of either sex (male or female) between 40-55 years.
- ❖ Compliance is high

Exclusion Criteria:

- Smokers.
- Patients with GIT, hepatic, cardiovascular, renal or endocrine disorders which can interfere with the absorption, metabolism and excretion of the study plant.
- Patients with any complication of diabetes mellitus.
- Pregnant patients.
- Where compliance is low
- Proper data entry not possible

Ethical Process

Written consents from volunteers were obtained after the study was being permitted by Institutional ethical committee (IEC). The study protocol was being explained to the volunteers in vernacular and a written brief description of the study in vernacular was provided to the volunteers. The research team sat together to see whether there was any conflict of interest and it was found to be nil. ICMR guidelines (2006) to conduct bio-medical researches involving human volunteers were followed.[11]

Selection of subjects

A group of 828 volunteers suffering from Type 2 diabetes were being selected from three villages, Balarampur and adjoining (semi-urban and urban), Gopali, Salua of Kharagpur Block and they were being screened for the study due to location advantages and on being permitted by village level authorities (panchayets) and municipal commissioners from the data available from local hospitals and health clinics. Of the 828 volunteers (both urban and rural) thus initially screened, 90 refused to give written consents and data of 83 people were not taken into account as they could not be monitored continuously due to lack of compliance such as frequent changes in life style and dietary intake patterns. 158 other volunteers demanded money for the participation and were eliminated. 7 persons suffered accident and their data eliminated in final calculations. Of the remaining 490 volunteers 350 were urban and 140 were rural. Of the 350 urban volunteers 300 were selected by random selection and of 140 rural volunteers 100 were selected also by a random selection due to financial constrains. Volunteers were living in close proximity with IIT Kharagpur. With these total of 400 volunteers, 20 groups (ten for the experimental and ten other for control) were formed again by a random selection basis (lottery). All the volunteers were briefed of maintaining the homogeneity and steps were taken to minimize the effects of other variables prior to the application of the control or treatment modes.

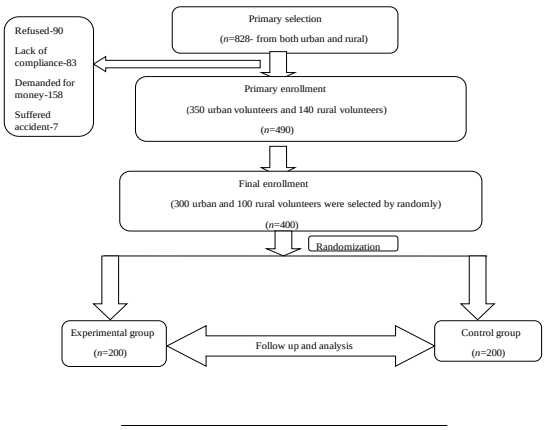

Figure 1: Flow chat representing the process of volunteers' selection

Selection of Local agents (LAs)

The selection of LAs was based on careful analysis of different factors, such as socioeconomic status, acceptability to the villagers, willingness to participate in the study, intelligence, ability to communicate with the volunteers regarding study objectives and methodology, scientific knowledge, ability to monitor dietary patterns correctly, able to collect and label blood samples etc. One LA was selected for every 20 volunteers and the LAs kept in regular contact with the volunteers and the research team. The diet in rural Bengal is a heterogeneous item, dependent on income among other factors; thus, proper measures were taken by the research team to maintain a homogeneous dietary pattern, as per the research objectives, among the volunteers. Life style pattern was carefully monitored to maintain the homogeneity.

Plants

Healthy plants of *Acalypha indica*, *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Musa sapientum*, *Mangifera indica*, *Murraya koenigii*, *Ocimum santum*, *Phyllanthus amarus* and *Tinospora cordifolia* were collected hygienically from the various localities of Indian Institute of Technology at Kharagpur (West Bengal, India) in the month of September 2007. All selected plants were botanically identified and authenticated by Dr. M. Senthilkumar, Plant Biotechnologist, Prathyusha Institute of Technology and Management, Chennai. A voucher specimen of each species was deposited in the herbarium, Prathyusha Institute of Technology and Management (Table 1). Collected healthy plant materials were adequately labeled. All selected plants were dried in indoor maintaining the hygienic conditions (temperature 27-30°C) for about 25-30 days. After complete drying (inspection), the dried materials of each specimen were grounded to fine powders using a domestic electric grinder (Product: GX 21, Bajaj appliances, Mumbai, India) and hygienically stored in sealed brown bottles at normal laboratory conditions to carry out further experimental part.

Preparation of Aqueous Extracts

After grinding the plant material in an electric grinder, the

Table 1: Plant species Used

Plant species	Family	Part used	Place of collection	Voucher sample
Acalypha Indica	Euphorbiaceae	Whole plant	Kharagpur	PITAM/CH/00025/2007
Allium Sativum	Alliaceae	Bulb	Kharagpur	PITAM/CH/00021/2007
Allium Cepa	Alliaceae	Bulb	Kharagpur	PITAM/CH/00010/2007
Azadirachta Indica	Meliaceae	Leaves	Kharagpur	PITAM/CH/00015/2007
Musa Sapientum	Musaceae	Flowers	Kharagpur	PITAM/CH/00017/2007
Mangifera Indica	Ancardiaceae	Stem barks	Kharagpur	PITAM/CH/00007/2007
Murraya Koenigii	Rutaceae	Leaves	Kharagpur	PITAM/CH/00009/2007
Ocimum Sanctum	Lamiaceae	Leaves	Kharagpur	PITAM/CH/00018/2007
Phyllanthus Amarus	Euphorbiaceae	Whole plant	Kharagpur	PITAM/CH/00011/2007
Tinospora Cordifolia	Meninspermaceae	Leaves	Kharagpur	PITAM/CH/00013/2007

plant powders (500 g) was macerated with one liter of Millipore water in a sterilized glass container and stirred intermittently and then left overnight under hygienic condition. After maceration, it was filtered through Whatmann filter paper (110 mm) and the filtrate was separated. After filtration the aqueous filtrate was stored aseptically in an air-tight container at 4°C.[12] The aqueous extract was used for human study in the experimentation process. Same procedure was followed for all selected plants to prepare their respective aqueous extracts.

Study description

The plant extracts as per documentation in Ayurvedic, tribal and folklore medicine were given to volunteers selected for the study. The experimental group volunteers received the extracts for consumption in the dose as being consumed by local tribes/populace, 5ml/day. Volunteers in control group received placebo (only water) from the research team for 2 months. The changes in the desired Blood Biochemistry Parameters (BBP) notably, fasting blood glucose (FBS), triglycerides (TG), total cholesterol (TC), high-density-lipoprotein (HDL), low-density-lipoprotein (LDL) and very low-density-lipoprotein (VLDL) in 12 hours fasting conditions were observed before and after the end of the study. Blood samples were collected before the initiation of the study to observe the baseline biochemical profiles of the volunteers and then at a monthly basis by professionals not related to the work under aseptic techniques and both clotted and un-clotted samples were duly labeled and immediately send to the Herbal medicine laboratory at School of Medical Science and Technology and BC Roy Technology Hospital, IIT Kharagpur. Blood samples were analyzed as per instruction sheets for manual assays given by Boehringer Mannheim Limited (1983) and the instrument used was photometer 4010 (Boehringer, Germany). Final results were statistically analyzed to see whether any statistical significance existed or not in order to derive any conclusion.

Collection of blood samples

The selected subjects (patients and controls) were medically examined and asked to present themselves on a specified date for blood tests. They were requested to come with fasting (no food before 12 hours). Blood samples (3-5 ml) were drawn from each patient and control subject by vene-puncture through plastic disposable syringes. The blood samples were collected in clean oven dried glass bottles which were previously rinsed with 1% sodium fluoride, 3% potassium oxalate solution to prevent coagulation. The plasma was separated after centrifugation. 12 hours fasting values were taken initially and at monthly intervals for two months. Measurement TC, HDL, LDL, VLDL, TG and FBS was done by standard methods as depicted by Boehringer Mannheim (Instruction sheets for manual assays GmbH Diagnostica, Catalogue no. 400 971, catalogue no.543 004, catalogue no.124 095, Catalogue no 124 966, Catalogue no.263 826, catalogue no. 123 919) and by reagents supplied by local vendors of Boehringer to meet the standard quality at monthly intervals. The other blood bio-chemical parameters were also evaluated before and after the experiment as per standard protocol of Boehringer (Boehringer Mannheim; Instruction sheets for manual assays GmbH Diagnostica).

Statistical analysis

All values were expressed as Mean ± Standard Deviation (S.D). The results of the BBPs were statistically analyzed using Windows-based SPSS statistical package (version 10.0; SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $p < 0.05$.

Results

The clinical, anthropometrical and biochemical evaluations were performed in all volunteers prior to the study. There were a total of 216 men and 184 women recruited to the study. The mean (±SD) age of the volunteers was 50.79± 4.2, mean weight was 76.3± 2.8 and mean body mass index (BMI) was 27.5±2.6 Kg/m^2. At the end of the study, there were no significant changes recorded for weight or BMI, which were 76.3±1.6 and 27.2 ± 2.4 Kg/m^2, respectively. Clinical parameters were also evaluated at the end of the study. Although some changes were noted in blood lipid and sugar levels, hemoglobin and in blood urea and serum bilirubin, these differences did not reach statistical significance. Anthropometric, clinical, and biochemical data were given in Table 2. Changes in TC, TG, HDL, LDL, VLDL and FBS were summarized in Table 3. There were significant changes observed biochemical parameters in experimental group. Fasting blood sugar level was lowered by *Mangifera indica* (136±14 to 130±12 mg/dl, p=0.02),

Table 2: Anthropometrical, Clinical and Biochemical data for the experimental and control groups at the end of 2 month study

Parameters	**Experimental Group Group (*n*=200)**	**Control Group Group (*n*=200)**
Age (years)	50.79 ± 4.6	50.56± 5.2
Male (*n)*	106	110
Female (*n*)	94	90
Weight (kg)	76.7 ± 2.6	76.2± 2.8
BMI (Kg/ m^2)	27.8 ± 2.3	28.3± 2.0
SBP (mmHg)	122±14	118±10
DBP (mmHg)	80±4	76±6
Serum Bilrubin (mg/dl)	0.6±0.8	0.7±0.6
Hemoglobin (g %)	13.5±0.6	13.4±0.4
Serum GPT (IU/L)	35±3.2	33±2.8
Blood Urea (mg/dl)	26±5.2	25±4.6
Serum Creatinine (mg/dl)	2.0±0.7	2.0±0.4
Serum Uric Acid (mg/dl)	5.5±0.6	5.4±0.7
Serum GOT (IU/L)	33±2.5	32±0.7
TC (mg/dl)	142± 10	154± 12
TG (mg/dl)	105± 5	135±7
HDL (mg/dl)	33± 4	35±3
LDL (mg/dl)	85±6	87±8
VLDL (mg/dl)	24±6	27±6
FBS (mg/dl)	131±8	135±7

Values are expressed as the mean ± SD. BMI-body mass index, SBP-systolic blood pressure, DBP-Diastolic blood pressure, TC-total cholesterol, TG-triglycerides, HDL-high density lipoprotein, LDL-low density lipoprotein, VLDL-very low density lipoprotein, FBS-fasting blood sugar GPT-glutamic pyruvic transaminase, GOT-glutamic oxaloacetic transaminase.

Table 3: Effects of aqueous plant extracts on biochemical parameters in experimental and control groups

Plants	**TC** (mg/dl)	**HDL** (mg/dl)	**LDL** (mg/dl)	**VLDL**(mg/dl)	**TG** (mg/dl)	**FBS** (mg/dl)
Acalypha Indica						
Control	152±14	33±5	92±17	27±9	135±1	152±11
Experimental	151±15	33±7	89±19	27±8	132±17	149±11
Allium Sativum						
Control	147±12	31±7	95±11	21±9	105±12	134±16
Experimental	144±18	32±8	92±09	20±8	99±12	131±12
Allium Cepa						
Control	145±18	28±9	99±14	18±6	90±13	138±21
Experimental	145±19	27± 6	102±12	15±9*	77±15*	135±14
Azadirachta Indica						
Control	132±17	22±6	90±12	20±5	100±8	125±12
Experimental	130±15	22±4	91±16	18±5	95±15	120±9*
Musa sapientum						
Control	142±14	22±5	102±7	18±6	90±14	137±21
Experimental	142±15	22±4	102±8	18±9	92±11	134±14
Mangifera indica						
Control	130±11	22±7	83±15	19±5	95±11	136±14
Experimental	124±19*	22±6	83±15	19±7	95±12	130±12*
Murraya Koenigii						
Control	135±14	26±9	89±14	20±6	100±8	134±9
Experimental	133±15	27±8	86±13	17±9*	100±9	129±10*
Ocimum Sanctum						
Control	142±14	25±3	91±14	26±5	130±21	135±19
Experimental	137±15*	27±4*	85±19*	25±8	127±15	132±14
Phyllanthus Amarus						
Control	128±18	22±5	85±17	21±5	105±13	144±12
Experimental	121±18 *	22±4	85±13	19±8	97±15*	138±10
Tinospora Cordifolia						
Control	132±15	24±6	84±14	24±5	125±13	137±19
Experimental	130±15	24±5	83±13	23±8	122±15	135±14

Values are given as the mean ± SD. *$P < 0.05$ compared with control group. TC-Total cholesterol, HDL-High density-lipoprotein, LDL- Low density-lipoprotein, VLDL- Very Low density-lipoprotein, TG- Triglycerides, FBS- Fasting blood sugar.

Murraya koenigii (134±9 to 129±10 mg/dl, p=0.03) and *Azadirachta indica* (125±12 to 120±9 mg/dl, p=0.03) in Type 2 diabetic patients. *Ocimum sanctum*s not only lower the total cholesterol (142±14 to 137±15 mg/dl, p=0.03) and LDL (91±14 to 85±19 mg/dl, p=0.03) level but also increase HDL (25±3 to 27±4 mg/dl, p=0.03) level Type 2 diabetic patients. In addition, *Allium cepa*, *Mangifera indica, Murraya koenigii* and *Phyllathus amarus* showed significant (p=0.03) reduction in TG, TC, and VLDL levels Type 2 diabetic patients. Age-specific data analysis in both the experimental and control groups showed no significant variations with age and therefore data in both groups were analyzed as a whole. In addition, there were no significant effects of gender observed.

Discussion

The control of blood glucose level is very important for patients suffering from Type 2 diabetes and for those who are at a high risk of developing it. When treating patients with diabetes, the basis of treatment should focus on motivating patients to pursue a healthier lifestyle, which has a major impact on progression of the disease. Unfortunately, physicians are usually not successful in controlling type 2 diabetes by dietary modification, exercise, and weight loss alone, and rely on pharmaceutical agents to control the disorder.[4] India has a record of very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. Indian traditional communities like tribal and primitive populations are frequently using the crude extracts of local plants for medicinal purposes. Plant based therapy are marked due to its low cast, easy availability and based on generation to generation knowledge.[13,14] Large number of Indian medicinal plants have been reported for their anti-diabetic and hypolipidemic effects in animal models.[15]

In the current study aqueous extracts of selected plants exhibited favorable anti-diabetic as well as hypolipidemic potentials in Type 2 diabetic patients. Aqueous extract of *Mangifera indica*, *Murraya koenigii* and *Azadirachta indica* showed reduction of fasting blood sugar level. The possible reason for the hypoglycemic effect can be attributed to the reduction in the carbohydrate absorption from the gut.

Since, lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in Type 2 Diabetes. High level of TC and LDL are major coronary risk factors.[16] Further, some studies suggested that TG itself is independently related to coronary heart disease.[17,18] The abnormalities in lipid metabolism lead to elevation in the levels of serum lipid and lipoprotein that in turn play an important role in occurrence of premature and severe atherosclerosis, which affects patients with diabetes.[19] Hence, measurements of biochemical parameters are necessary to prevent complications in diabetic condition.

In the present study, aqueous extract of *Phyllanthus amarus*, and *Mangifera indica* decreased total cholesterol level in Type 2 diabetic patients. Aqueous extract of *Ocimum santum* not only lower total cholesterol and LDL level but also increase HDL (cardioprotective lipid) in Type 2 Diabetic patients. In addition, aqueous extract of *Allium cepa* and *Murray koenigii* exhibited reduction of VLDL. Further, *Phyllanthus amarus* and *Allium cepa* showed an effective reduction of TG in Type 2 Diabetic patients. Therefore, aqueous extracts of *Mangifera indica, Murraya koenigii, Ocimum santum, Phyllanthus amarus, Allium cep* and *Azadirachta indica* have potential role in management of Type 2 Diabetes as well as to prevent formation of atherosclerosis and coronary heart disease in Type 2 Diabetic patients.

There are several studies regarding the use of herbal extract for the treatment of diabetes. The available literatures suggest that herbal extracts are well tolerated with negligible side effects during treatment of diabetes. Garlic (*Allium Sativum*) preparation used as an adjunct agent in treatment of arterial hypertension because of its hypolipidemic and antioxidant properties in patients.[20] Garlic (*Allium Sativum*) extract supplementation improved blood lipid profile, strengthens blood antioxidant potential and causes significant reduction in systolic and diastolic pressures in human volunteers.[21] Onion (*Allium cepa*) and garlic (*Allium Sativum*) exhibited anti-diabetic, antibiotic and hypocholestrolaemic actions and they had been used in the traditional medical practice of many cultures to treat cardiovascular and other disorders.[22,23] Aqueous and alcoholic extracts of *Azadirachta indica* showed significant hypoglycemic activity in Type 2 diabetic patients.[24] *Musa Sapientum* L. commonly known as '*banana*' is mainly used in Indian folk medicine for the treatment of Diabetes mellitus.[25] The aqueous extracts of stem bark of *Mangifera indica* possess antioxidant, anti-inflammatory and immunomodulatory effects[26] and showed protection against serum oxidative stress in elderly human volunteers.[27] *Murraya koenigii* leaves being used in Ayurvedic medicine and aqueous extract of *Murraya koenigii* leaves had hypoglycemic effect in normal and diabetic rabbits.[28] *Ocimum sanctum* and *Ocimum album* (holy basil) leaf extract showed hypoglycemic effects. Further, it could be used as adjunct therapy and drug treatment in mild to moderate Type 2 diabetes.[29] A study indicated that diabetic patients with foot ulcer on *Tinospora cordifolia* as an adjuvant therapy showed effectiveness in wound healing by its immunomodulation activities.[30]

The present study suggested that aqueous extracts of *Mangifera indica, Murraya koenigii, Ocimum santum, Phyllanthus amarus, Allium cep* and *Azadirachta indica* have potential role in management of Type 2 Diabetes as well as to prevent formation of atherosclerosis and coronary heart disease in Type 2 diabetic patients.

Conclusion

In conclusion, India is a developing country with a large portion of people suffering from DM and its complications. A large portion of the population lives in rural sectors with poor health infrastructures. The high costs for therapeutic treatment has compelled physicians to look for alternative

cost-effective methods to minimize complications associated with Type 2 diabetes. Therefore, medicinal plants and their products are more convenient for the treatment of Type 2 diabetes due to their easy availability, low cost, minimum side effects and greater acceptance amongst the users. This study indicated use of aqueous extracts of *Mangifera indica*, *Murraya koenigii*, *Ocimum santum*, *Phyllanthus amarus*, *Allium cep* and *Azadirachta indica* exhibited anti-diabetic as well as hypolipidaemic effects Type 2 diabetic patients.

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