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Anti-hyperglycaemic and anti-dyslipidaemic effect of dietary supplement of white *Ocimum Sanctum Linnean* before and after STZ-induced diabetes mellitus

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Abstract

This study was conducted to elucidate whether dietary treatment of white *Ocimum sanctum Linnean*. (OS) either before or after streptozotocin (STZ) - induced diabetes has anti-hyperglycemic and anti-dyslipidaemic action. Two series of experiments were performed. The first involved three groups of rats; one group was fed normal diet for 6 weeks, a second diabetic group received normal diet and a third diabetic group received 2 % dietary OS from 3 weeks after induction of diabetes for a period of 3 weeks. The second involved three groups of rats: one group was fed normal diet, a second group received or did not receive 2 % dietary white OS before induction of diabetes. Fasting blood glucose was determined before and after induction of diabetes. At the end of the study, arterial blood was collected to evaluate serum triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and creatinine. The results show that blood glucose was not significantly altered after 3 weeks of dietary supplement of white OS in diabetic rats. In contrast, elevated blood glucose after 3 weeks of diabetes was alleviated in diabetic rats pretreated with white OS. STZ-induced diabetes significantly raised serum triglyceride, total cholesterol, LDL-cholesterol, AST, ALT and creatinine. White OS supplementation, either before or after induction of diabetes, normalized lipid profile and creatinine and partially reduced an elevated serum AST and ALT. It can be concluded that STZ-induced hyperglycaemia can be ameliorated by pretreatment with white OS. Dietary supplement with OS either before or after diabetic induction reverses dyslipidaemia and renal glomerular filtration function, and partially protects liver function.

Key words: *diabetes mellitus, serum lipid, Ocimum sanctum Linnean.*

Introduction

Diabetes mellitus (DM) is a serious metabolic disease which has several complications including diabetic nephropathy, diabetic neuropathy, coronary heart disease and hypertension.[1] It has been estimated that by the year 2010, the prevalence of DM worldwide will reach approximately 240 million.[2] Patients with DM are more likely to develop and die from microvascular and macrovascular complications than the non-diabetic population.[3] There is usually an association between coronary heart disease or atherosclerosis and dyslipidaemia.[4,5] Dyslipidaemia is a frequent complication of DM and is characterized by low levels of HDL-cholesterol and high levels of LDL-cholesterol and triglyceride. Several groups of hypoglycaemic drugs are currently available to treat DM. However, their toxic side effects and sometimes diminution in response after prolonged use are problematic. Management of DM to avoid these problems is still a major challenge. There is an ongoing search for natural products with anti-hyperglycaemic and anti-dyslipidaemic activities with minimal side effects. There are several kinds of medicinal plants in Thailand which have been reported to exert anti-hyperglycaemic and/or anti-dyslipidaemic actions.[6] Among them, *Ocimum sanctum Linnean.* (OS) is very promising since it is routinely used as a vegetable and also for the treatment of DM by local people in various countries including India and Thailand. Several studies have demonstrated that OS possesses anti-hyperglycaemic and/or anti-dyslipidaemic effect in normal and DM animals.[7-10] Preliminary studies in our laboratory have shown that white OS exerts hypoglycemic action in normal rats whereas red OS was without this effect. We have investigated whether supplementation of diet with white OS can retard dyslipidaemia and hyperglycaemia in diabetes.

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Materials and methods

Animal preparation

Male Wistar rats weighing between 180-220 g from Animal Center, Salaya Campus, Mahidol University, were used in the

Table 1: Changes in body weight, food intake and fasting blood glucose in rats fed with or without white *Ocimum sanctum* Linnean. (OS) during the last three weeks of the study.

	Before feeding with or without OS		After feeding with or without OS	
	1 wk	3 wk	1 wk	3 wk
Body weight (g)				
Group 1	200.00 ± 3.32	274.00 ± 5.05	300.86 ± 8.02**	351.14 ± 10.69***
Group II	212.57 ± 4.73	221.71 ± 6.55	233.14 ± 7.23*	231.14 ± 6.95*
Group III	180.86 ± 4.20	194.57 ± 4.48	196.57 ± 4.47	213.43 ± 6.19**
Food Intake (g/24 hr)				
Group 1	20.57 ± 1.36	22.29 ± 2.28	22.29 ± 2.69	24.14 ± 1.35
Group II	35.00 ± 3.31	41.86 ± 2.62	43.71 ± 1.30	45.86 ± 1.10
Group III	35.28 ± 3.04	37.43 ± 2.24	37.86 ± 2.20	37.14 ± 2.99
Blood glucose (mg/dl)				
Group 1	84.71 ± 3.01	99.00 ± 4.92	105.14 ± 4.05	100.43 ± 1.66
Group II	250.71 ± 19.20	318.14 ± 24.18	310.86 ± 25.20	357.28 ± 26.30
Group III	230.29 ± 28.47	279.28 ± 23.08	305.86 ± 27.82	326.00 ± 28.46

Values are shown as mean ± SEM.
group I = normal control rats , group II = diabetic rats fed normal diet, group III = diabetic rats fed with white *Ocimum sanctum Linnean*.
* significant difference comparing to the third week before white OS treatment (*P<05, **P<0.01, ***P<0.001).

Table 2: Changes in serum lipid profile, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and serum creatinine (Cr) in rats fed with or without white *Ocimum sanctum Linnean*. during the last three weeks of this study.

	Group I	Group II	Group III
Triglyceride (mg/dl)	95.62 ± 11.49	219.02 ± 24.99*	108.98 ± 14.88 #
Total Cholesterol (mg/dl)	86.29 ± 8.48	208.27 ± 32.65***	97.84 ± 6.52 #
HDL Cholesterol (mg/dl)	46.86 ± 5.79	39.36 ± 3.59	50.76 ± 3.51
LDL Cholesterol (mg/dl)	27.84 ± 1.97	41.76 ± 4.61*	27.56 ± 3.54#
AST(unit/L)	30.79 ± 2.16	57.50 ± 4.07*	45.79 ± 3.72a
ALT (unit/L)	23.00 ± 2.68	60.71 ± 3.83***	44.29 ± 4.55#b
Cr (mg/dl)	1.04 ± 0.04	1.96 ± 0.18**	1.21 ± 0.08##

Data are presented as mean ± SEM
Abbreviations for each group are shown in table I
* significant difference comparing to group I (* P<0.05 , ** P<0.01 ,*** P<0.001).
# significant difference comparing to group II (# P< 0.05 , ## P<0.01)
a ,b significant difference comparing to group I at P< 0.05 and P<0.01 respectively

study. Rats were housed in a 12-hr light-dark cycle at 25 ± 2 °C and fed normal rat food and tap water *ad libitum*. All animals were cared for in accordance with the principles and guidelines of the Institutional Animal Ethics Committee of Rangsit University, which is under The National Council of Thailand for Animal Care. Induction of diabetes was carried out by intraperitoneal injection of STZ (Sigma, St Louis, MO, USA) dissolved in citrate buffer pH 4.5 at a dose of 70 mg/kg bodyweight. Two days after STZ injection blood glucose was measured and only those rats with fasting blood glucose >170 mg/dl were included in the study

Preparation of white *Ocimum sanctum* Linnean

Fresh leaves of white OS obtained from The Ministry of Public Health, Thailand, were washed in tap water and then left to dry at room temperature for 2-3 days. The dried leaves were then ground to fine powder in a mixer. The dried leaf powder was then added to the diet to makeup 2 % of the diet.

Experimental design

Two series of experiments were performed.

Series I: ***The effect of dietary supplement of white OS on blood glucose and serum lipid 3 weeks after induction of diabetes***

Three groups of 7 rats were used as follows:
Group I**:** Control rats fed with normal diet for 6 weeks. The normal diet contained protein 23.5 %, fat 4.4 %, fibre 4.9 %, mineral and vitamin 6.4 %, moisture 12 % and carbohydrate 46.8 % with added corn starch 2 %.
Group II: Diabetic rats fed with normal diet throughout 6 weeks.
Group III: Diabetic rats fed with normal diet for 3 weeks and then switched to normal diet with 2 % white OS which replaced the corn starch.

Body weight, food consumption and fasting blood glucose were determined at the first and third week after the start of

Table 3 The alterations of body weight, food intake and fasting blood glucose in rats pretreated with or without white *Ocimum sanctum* Linnean. (OS) for three weeks before diabetic induction.

	Before induction of Diabetes		After induction of Diabetes	
	1 wk	3 wk	1 wk	3 wk
Body weight (g)				
Group A	200.00 ± 3.32	274.00 ± 5.05	300.86 ± 8.02**	351.14 ± 10.69***
Group B	193.83 ± 2.76	238.00 ± 1.89	231.003.68	232.67 ± 4.28
Group C	198.00 ± 5.93	249.67 ± 6.39	240.00 ± 7.90	245.67 ± 11.51
Food Intake (g/24 hr)				
Group A	20.57 ± 1.36	22.29 ± 2.28	22.29 ± 2.69	24.14 ± 1.35
Group B	23.83 ± 1.19	28.00 ± 1.63	24.83 ± 2.36	48.83 ± 5.57**
Group C	27.00 ± 2.57	28.83 ± 2.55	24.33 ± 4.68	38.83 ± 1.14*
Blood glucose (mg/dl)				
Group A	84.71 ± 3.01	99.00 ± 4.92	105.14 ± 4.05	100.43 ± 1.66
Group B	89.67 ± 1.64	90.83 ± 1.00	260.50 ± 14.37***	336.33 ± 10.76***
Group C	85.67 ± 4.37	89.00 ± 1.17	211.83 ± 24.36**	243.67 ± 29.29**[a]

Values are given as mean ± SEM.
group A = normal control rats.
group B = diabetic rats without white *Ocimum sanctum Linnean* pretreatment
group C = diabetic rats with white *Ocimum sanctum Linnean* pretreatment and continued until end of experiment
* significant difference comparing to the third week before diabetic induction (*P<0.05 , **P<0.01, *** P<0.001)
[a] significant difference comparing to group B at the same period at P< 0.05

Table 4 Changes of serum lipid profile, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and serum creatinine (Cr) in rats pretreated with or without white *Ocimum sanctum Linnean* (OS) for 3 weeks before induction of diabetes.

	Group A	Group B	Group C
Triglyceride (mg/dl)	95.62 ± 11.49	224.37 ± 17.97***	114.49 ± 20.31[##]
Total Cholesterol (mg/dl)	86.29 ± 8.48	114.72 ± 5.60*	74.96 ± 6.79[##]
HDL Cholesterol (mg/dl)	46.86 ± 5.79	40.19 ± 2.07	39.08 ± 3.12
LDL Cholesterol (mg/dl)	27.84 ± 1.97	40.39 ± 1.23**	34.26 ± 2.06[#]
AST unit/L	30.79 ± 2.16	72.58 ± 3.22***	40.29 ± 2.39[####a]
ALT (unit/L)	23.00 ± 2.68	63.17 ± 1.92 ***	38.57 ± 4.77 [####a]
Cr (mg/dl)	1.04 ± 0.04	1.93 ± 0.25***	0.88 ± 0.07[##]

Data are presented as mean ± SEM
Abbreviations for each group are shown in table 2
* significant difference comparing to group A (*P<0.05 , ** P<0.01, *** P<0.001)
[#], [##], [###] significant difference comparing to group B at P<0.05, P<0.01 and P<0.001 respectively.
[a] significant difference comparing to group A at P<0.05.

the study. After 1 and 3 weeks of either white OS or normal diet treatment, body weight, food consumption and fasting blood glucose were again measured. At the end of the experiments rats were fasted overnight and then arterial blood was collected from abdominal aorta to determine serum lipid profile (triglyceride, cholesterol, HDL-cholesterol and LDL-cholesterol). To determine whether dietary supplementation with white OS affects liver and kidney function, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and serum creatinine were measured.

Series II: ***Effect of pretreatment with white OS on blood glucose and serum lipid in diabetic rats***

Three groups of seven rats each were used as follows:

Group A: Control rats treated with normal diet for 6 weeks.
Group B: Rats were fed with normal diet for 3 weeks, and then injected with STZ. Normal diet was supplied for remainder of the study.
Group C: As for Group B except 2 % of white OS in diet was provided both before and after induction of diabetes.
In order to prevent experimental error owing to STZ injection in group B and C, only rats with fasting blood glucose >140 mg/dl were included in the study.

Body weight, food intake and fasting blood glucose were determined at the first and third week after the beginning of the experiment, and then again after 1 and 3 weeks of diabetes. At the end of the experiment rats were fasted

overnight and blood was collected from the abdominal aorta to determine serum lipid profile, AST, ALT and creatinine.

Biochemical assay

Fasting blood glucose was determined by blood glucose strip (Medisense UK Ltd, Abbott Lab, UK). Serum lipid profile, creatinine, AST and ALT were determined using an enzymatic kit (Human, Gesellschaft Für Biochemica und Diagnostica mbH, Germany)

Data and Statistical Analysis

Data are presented as mean ± SEM. The results were analyzed for statistical significance by ANOVA using SPSS version 10. Significant difference was accepted at the $P < 0.05$.

Results

Effect of dietary supplement of white OS on blood glucose and serum lipid after 3 weeks of diabetes

Table 1 shows the changes in body weight, food intake and fasting blood glucose in the rats before and after being fed with either normal diet or diet with white OS after 3 weeks. There was no significant change in food intake and blood glucose in both groups of diabetic rats with or without white OS. Also, no significant difference in blood glucose was noted in both groups of diabetic rats with or without white OS.

Serum lipid profiles of rats with or without white OS during the last 3 weeks are shown in Table 2. Triglyceride and total cholesterol were significantly raised from 95.62 ± 11.49 and 86.29 ± 8.48 in normal control to 219.02 ± 24.99 ($P < 0.05$) and 208.27 ± 32.65 mg/dl ($P < 0.01$), respectively in diabetic rats fed normal diet. Three weeks of dietary supplementation of white OS to diabetic rats returned the high level of both triglyceride and cholesterol to a level that was not significant by difference from normal control rats (108.98 ± 14.88 and 97.84 ± 6.52 mg/dl, respectively). HDL-cholesterol was lowered in diabetic rats (39.36 ± 3.59) but the level was not significant by difference from normal control rats (46.86 ± 5.79). White OS feeding to diabetic rats slightly raised HDL-cholesterol but the magnitude was not statistically significant by difference from normal diabetic rats. The elevation of LDL-cholesterol in normal diabetic rats (41.76 ± 4.61 mg/dl) was normalized after 3 weeks of white OS supplementation ($P <0.05$). White OS supplementation alleviated an augmentation of both serum AST and ALT but the level remained higher than that of normal control rats. White OS feeding reduced an elevation of serum creatinine in diabetic rats fed normal diet (1.96 ± 0.18 mg/dl) to the level that was statistically not significantly different from normal control rats (1.21 ± 0.08 mg/dl).

Effect of pretreatment of dietary white OS on blood glucose and serum lipid in diabetic rats

The alterations of body weight, food intake and blood glucose in rats pretreated with or without white OS before diabetic induction are illustrated in Table 3. Although there was no significant change in body weight there was an increased food intake after the onset of diabetes. Blood glucose was significantly raised from 90.83 ± 1.00 to 260.50 ± 14.37 mg/dl ($P < 0.001$) and 89.00 ± 1.17 to 211.83 ± 24.36 mg/dl ($P < 0.01$) one week after STZ injection in rats pretreated with or without white OS, respectively. There was only one rat from each group of diabetic rats in which fasting blood glucose was lower than 140 mg/dl after STZ injection and hence, was excluded from the study. The magnitude of blood glucose elevation after 1 week of STZ injection in rats pretreated with white OS was slightly lower than that of another group but the level was not statistically significantly different. Three weeks after induction of diabetes, blood glucose in rats pretreated with white OS was significantly lower than that of diabetic rats without white OS treatment ($P <0.05$).

Similar to the first series of experiments, pretreatment with white OS for 3 weeks before induction of diabetes reduced the elevated levels of triglyceride, total cholesterol and LDL-cholesterol to a level which was statistically not significantly different from normal control rats (table 2). Pretreatment with white OS for 3 weeks before induction of diabetes alleviated an augmentation of AST, ALT and normalized serum creatinine.

Discussion

Ocimum sanctum Linnean (OS) is a medicinal plant distributed mainly in the tropical and subtropical regions including Thailand. Besides being widely used as a vegetable it has also been used as an indigenous medicine in several other countries in Asia and Africa[11], particularly for its hypoglycaemic and anti-dyslipidaemic effects in diabetes.[7-10] However, these effects were displayed only in very short-term studies. However, DM is a chronic disease and most diabetic patients do not know whether they are diabetic until symptoms such as diuresis, polydipsia, polyphagia present themselves.

Whether OS normalizes dyslipidaemia and/or hyperglycaemia after longer periods of diabetes is not known. From the present results, 3 weeks of dietary supplement with white OS to the rats after induction of diabetes for 3 weeks, had no significant effect on blood glucose. This experimental result was quite different from other studies[7,12] which showed a glucose-lowering action of OS. This inconsistency of data could be for a variety of reasons. The study of Vats et al[12] and Chattopadhyay and his colleagues[7] orally fed diabetic rats with the aqueous extract of OS leaves whereas dietary supplement of crude OS leaves was used in our study. The aqueous extract of OS may have much more active ingredients than crude dry leaves. However, it is quite difficult to mix extracts of OS into the diet since it was a thick paste and is sticky in nature. Furthermore, daily oral feeding with the aqueous extract of OS for 3 weeks was likely to be stressful to the rats. Of particular interest is that oral feeding of the aqueous extract of OS begun during an early period after STZ

or alloxan injection whereas it was given after induction of diabetes for 3 weeks in the present study. Moreover it has been shown that hypoglycemic activity of aqueous extract of OS is different in rats with different degrees of hyperglycaemia induced by STZ or alloxan injection. The greater the degree of hyperglycaemia the lesser the hypoglycemic effect of aqueous extract of OS.[12,13] In this study, the blood glucose level in diabetic rats before white OS treatment was approximately 300 mg/dl whereas in other studies it was 140-300 mg/dl. It has been suggested that the anti-hyperglycaemic effect of OS is at least partially dependent on insulin release from β–cells as shown by a greater anti-hyperglycaemic activity in mild hyperglycaemia and lower response in moderate hyperglycaemia.[13] Prolonged hyperglycaemia along with severe hyperglycaemia as occurred in the present study, may be the cause of failure of glucose lowering action of OS.

Though dietary supplementation of white OS after induction of diabetes had no glucose lowering effect, it reduced hyperglycaemia in rats pretreated with white OS. The reason why pretreatment with white OS in diabetic rats resulted in the reduction of hyperglycaemia and treatment with white OS 3 weeks after induction of DM is unclear. It may be that pretreatment of white OS before induction of diabetes caused a gradual accumulation of active ingredients which may have been enough to act against STZ-induced β-cells causing damage by increasing islet superoxide dismutase activity,[14] and then alleviating hyperglycaemia after STZ injection.

One of the most critical complications of DM is atherosclerosis and coronary heart disease which are the result of abnormal lipid metabolism.[4] It has been reported that the lipid profile of DM is characterized by low levels of HDL-cholesterol, and elevated LDL-cholesterol and triglyceride levels.[4,15] This combination is often termed diabetic dyslipidaemia. LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of arteries. A rise in LDL may cause deposition of cholesterol in arterial walls and hence promote atherosclerosis and coronary heart disease. In the present study, not only pre-treatment but also post-treatment with white OS normalized dyslipidaemia in diabetic rats. This observation indicates that dietary supplement of white OS either before or after induction of diabetes may be effective at preventing and/or ameliorating atherosclerosis and coronary heart disease. It is interesting to note that dietary supplementation with white OS exerted different effects on blood glucose but showed the same effect on lipid profile. This indicates that recovery of dyslipidaemia in diabetic rats treated with white OS is independent of blood glucose levels and that glucose lowering activity and anti-dyslipidemic activity may be provided by different active ingredients in OS. OS leaves have been reported to contain several chemicals including ursolic acid, apigenin, luteolin, orientin, moludistin.[16] It is widely known that OS leaves are rich in essential oils, particularly eugenol.[17] Currently no experimental data has clearly supported an anti-hyperglycaemic and anti-dyslipidemic mechanism for white OS. Moreover, it is not clear which of its constituents or combination of these constituents are responsible for the anti-hyperglycemic and anti-dyslipidaemic effects.

In the living system, the liver and kidney are highly sensitive to toxic or foreign agents. It is widely known that renal glomerular capillaries and hepatic cells damage are often found in DM. To assess experimental liver damage, serum AST and ALT, which are more abundant in liver, were determined. It was observed that dietary supplementation with white OS either before or after induction of diabetes partially reversed elevated serum AST and ALT, implying that the magnitude of hepatic cell damage was reduced. Eugenol, a major essential oil in OS, may contribute to this action since it has been shown that oral administration of eugenol reduced iron-induced hepatic damage.[18] Dietary treatment of OS also normalized a high level of serum creatinine in diabetic rats, indicating its protective effect on renal glomerular filtration ability.

In summary, dietary supplementation with white OS for 3 weeks after 3 weeks of diabetes had no significant effect on blood glucose. In contrast, pre-treatment with white OS for 3 weeks before induction of diabetes reduced STZ-induced hyperglycaemia. Dietary treatment of white OS either before or after induction of diabetes significantly reduced the elevated serum lipid profile, serum enzyme AST, ALT and creatinine. The findings from this study indicate that STZ-induced hyperglycaemia can be ameliorated by pre-treatment with white OS. Dyslipidaemia is normalized in diabetic rats supplemented with white OS either before or after induction of diabetes. White OS also partially protects hepatic cell damage and reverses renal glomerular filtration dysfunction in diabetic rats.

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