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Antidiabetic, antihypercholesterolaemic and antioxidant effect of *Ocimum sanctum* (Linn) seed oil

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Antihyperlipidaemic and antioxidant effect of *Ocimum sanctum* Linn. seed oil (OSSO) was investigated in rabbits. Administration of OSSO (0.8 g/kg body weight/day) for four weeks, in cholesterol (100 mg/kg body weight/day) fed rabbits significantly decreased serum cholesterol, triacylglycerol and LDL-+VLDL-cholesterol as compared to untreated cholesterol fed group. There was significant fall in atherogenic index in OSSO treated group. In addition, treatment with OSSO decreased lipid peroxidation and increased reduced glutathione content in blood. Antidiabetic effect of *O. sanctum* seed oil was evaluated in alloxan diabetic rabbits. Two weeks treatment of diabetic rabbits with OSSO (0.8 gm/kg/day) showed no significant hypoglycaemic effect. Results of the present study show that OSSO has hypocholesterolaemic and antioxidant effects but it does not have antidiabetic effect.

Keywords: Antioxidant activity, Hypocholesterolaemic agents, *Ocimum sanctum*, Seed oil.

Ocimum sanctum (OS, Family-Labiatae), known as Tulsi in Hindi and Holy basil in English has been extensively used in Ayurvedic system of medicine. Each part of the plant has medicinal value. The juice of the stem and leaves is diaphoretic and expectorant. It relieves earache and is used to treat skin disorders[1]. Efficacy of OS leaves in decreasing blood glucose has also been reported[2,3]. The fixed oil obtained from seeds of OS, is rich in unsaturated fatty acids, it contains (%) palmitic acid (11.69), stearic acid (3.19), oleic acid (13.82), linoleic acid (52.24) and linolenic acid 16.63%[4]. OS seed oil has anti-inflammatory activity which is attributed to linoleic acid present in the oil[4,5]. It was reported to have chemoprotective activity in mice[6]. This effect of OS seed oil is due to scavenging and detoxifying effect on reactive carcinogenic species produced by carcinogen methylcholanthrene. It also had antipyretic, analgesic, immunomodulatory, antiarthritic and hypoglycaemic activity[3,7-9]. Hypolipidaemic and antioxidant activity of OS leaves have also been reported. OS leaf powder supplementation at 1 to 2% dose levels showed significant hypolipidaemic effect in rabbits[10]. Aqueous extract of leaves has been found to protect mouse against radiation lethality and to possess significant antioxidant effect[11-13]. However, so far to our knowledge there are no reports on effect of OS seed oil on serum lipid profile and oxidative stress and there is only one report on hypoglycaemic effect of OS seeds[3]. Therefore, the present study is aimed at assessing antidiabetic, antihypercholesterolemic and antioxidant effects of OS seed oil.

Materials and Methods

Plant material—Dried seeds of *O. sanctum* collected locally and authenticated by a resident botanist at the Department of Genetics, Indian Agricultural Research Institute, Pusa, New Delhi, were crushed and cold macerated in petroleum ether for three days. The petroleum ether was evaporated from the extract and oil was filtered to clarity. It was then stored at room temperature in amber-coloured light-protected bottles for experimental use. The yield of the oil was 22% v/w.

Animals and experimental design—Male albino rabbits (1-1.5 kg) were used in the study. They were kept in a 12:12 hr L:D cycle and temperature (22°±2°C) controlled conditions. Standard laboratory pellet diet (Hindustan Lever, Bombay) and water were given *ad libitum*. The care of the animals was as per the 'Guidelines for the Care and Use of Animals

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in Scientific Research' prepared by the Indian National Science Academy, New Delhi[14].

Assessment of hypocholesterolaemic and antioxidant activity—Hypercholesterolaemia was induced by the method of Ratnakar & Murthy[15]. Rabbits were divided into 3 groups of 5 each and fed for 5 weeks as follows:

Group I – Normal diet
Group II – Normal diet + crystalline cholesterol (Loba Chemie) 100 mg/kg body weight/day suspended in groundnut oil for 5 weeks.
Group III – Normal diet + cholesterol as above for 5 weeks + OSSO from 2nd to 5th week (0.8 g/kg body weight/day, po).

Blood samples were collected from marginal ear vein of overnight fasted rabbits at 0, 1st and 5th week.

Assessment of hypoglycaemic activity

Induction of diabetes—Fresh solution of alloxan (80 mg/kg) in 0.5% saline, *p*H 4.5 was injected in marginal ear vein of overnight fasted rabbits. Fasting blood glucose (FBG) was estimated at 5 days interval upto 30 days. Rabbits with FBG above 120 mg/dl and showing abnormal glucose tolerance were considered diabetic.

Oral glucose tolerance test (OGTT)—Fasting blood samples were collected and glucose was given to rabbits orally (2 g/kg body weight). Subsequent blood samples were collected at 1 and 2 hr for glucose estimation.

Experimental design—Rabbits were distributed into three groups

Group A: Healthy control
Group B: Untreated diabetic rabbits
Group C: Diabetic rabbits treated with OSSO (0.8 gm/kg/day, po) for two weeks.

FBG and GTT were determined before starting treatment and at the end of 2nd week.

Estimation of biochemical parameters—Glucose in blood, total cholesterol (TC), HDL-cholesterol (HDL-C), triacylglycerol (TAG), in serum were estimated enzymatically using kits from Orthodiagnostics, Bombay. LDL-cholesterol (LDL-C) was calculated by Friedewald's formula[16]. Lipid peroxides were measured in serum as thiobarbituric acid reactive substances (TBARS) as per Satoh[17] and the reduced glutathione (GSH) content in blood was measured by the method of Beutler[18] using 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB).

Statistical analysis—All values were expressed as mean±SD using one way ANOVA analysis. The significance of differences between the mean of the treated and untreated groups were established by Tukey's test. $P<0.05$ was considered significant.

Results

Serum lipid levels—Cholesterol feeding increased total cholesterol, TAG, LDL- + VLDL-cholesterol, significantly at the end of 5th week ($P<0.001$) in animals of group II as compared to group I rabbits which were kept on standard pellet diet (Table 1). Rise in different serum lipid levels in group III rabbits were comparable to group II at the end of first week as both groups received similar amount of cholesterol.

Table 1—Effect of treatment with *Ocimum sanctum* seed oil on serum lipid levels of hypercholesterolaemic rabbits [Values are mean±SD from 5 animals in each group]

	Time (weeks)	Total Cholesterol (mg%)	HDL-C (mg%)	LDL- + VLDL-C (mg%)	TAG (mg%)	Atherogenic index HDL-C (LDL+VLDL-C/HDL-C)
Group I	0	42.6 ± 2.7	20.8 ± 3.3	22.4 ± 4.2	58 ± 6.2	1.07
(Healthy control)	1	43.9 ± 4.4	21.6 ± 4.0	22 ± 4.0	59 ± 7.2	1.02
	5	44.3 ± 3.1	20.0 ± 3.8	23.2 ± 3.7	60 ± 5.9	1.16
Group II	0	47.8 ± 5.3	21.2 ± 2.5	21.8 ± 5.4	64 ± 11	1.03
	1	116 ± 18.5	21.4 ± 2.4	93.8 ± 12.2	88.6 ± 13.5	4.38
	5	363 ± 69	22.0 ± 3.1	341 ± 39.4	204 ± 29.5	15.5
Group III	0	48 ± 8.1	22.0 ± 3.1	23.6 ± 15.2	67 ± 9.2	1.07
	1	120 ± 15.5	22.0 ± 2.5	94 ± 7.8	91 ± 9.1	4.27
	5	177 ± 20.7	23.8 ± 1.7	140 ± 20.8	107 ± 17	5.88
P value, Group III compared to Group II on 5th week		0.001	NS	0.001	0.001	

From 8th day onwards, group III animals received OSSO in addition to cholesterol feeding orally for four weeks. Treatment with OSSO along with cholesterol feeding for four weeks i.e. from 2nd to 5th week resulted in 51% fall in total cholesterol, 47% fall in TAG and 59% fall in LDL- + VLDL-cholesterol levels (Table 1), in group III compared to group II. There was no significant change in HDL-C in all the three groups. Atherogenic index (ratio of LDL- + VLDL-C to HDL-C) was nearly same in the 0 week in all the three groups. It increased in group II significantly. Group III has increased atherogenic index at the end of 5th week but the rise was significantly low as compared to group II (Table 1).

Serum TBARS and GSH levels—Group II has significantly higher serum TBARS levels at the end of 5th week as compared to group I (Fig. 1a). Group III rabbits have significantly lower serum TBARS as compared to group II, i.e. untreated group (P=0.001). Reduced glutathione was significantly low in group II as compared to group I and treatment increased its level in group III (Fig. 1b).

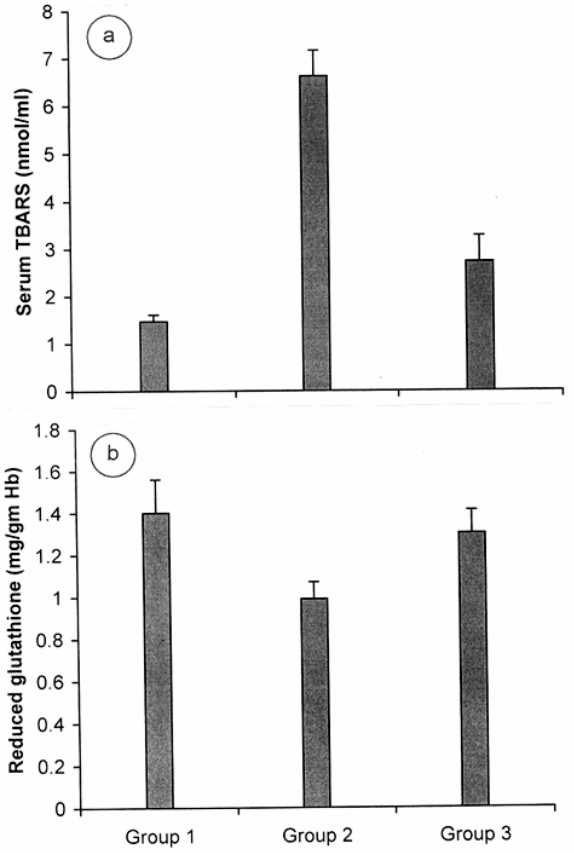

Fig. 1—Effect of *O. sanctum* seed oil on (a) serum TBARS levels, and (b) reduced glutathione levels in erythrocytes of hypercholesterolaemic rabbits

Blood glucose and glucose tolerance test—Treatment with OSSO for two weeks produced no significant fall in FBG or improvement in GTT in diabetic rabbits (Table 2).

Discussion

Hypercholesterolaemia and hypertriacylglycerolaemia are major risk factors for atherosclerosis and related occlusive vascular disease[19]. Clinical complications as atherosclerosis could be diminished and life prolonged when blood lipids are lowered by hypocholesterolaemic drugs[20,21].

The results of present study demonstrated that OS seed oil has lipid lowering effect. There was significant rise in serum cholesterol, LDL- + VLDL cholesterol, atherogenic index and TAG in group II rabbits which received cholesterol suspended in oil for 5 weeks. HDL-cholesterol did not increase. Similar changes in serum cholesterol, LDL- + VLDL-C and TAG upon cholesterol feeding have been reported[22,23]. Rise in TC, LDL- + VLDL-C and TAG was similar in groups II and III in first week. However, as group III received OS seed oil from 2nd week, there was significant fall in all these parameters (P=0.001). Similar improvement in lipid profile has also been observed with other plants[22-27]. Levels of TC, LDL + VLDL-C and TAG did not return to normal in group III, probably because treated group also received cholesterol in oil throughout the experiment. But significantly low levels of TC, LDL + VLDL-C, TAG and decreased atherogenic index in OS seed oil treated group as compared to untreated group confirm hypolipidaemic effect of OS seed oil.

Table 2—Effect of OSSO on fasting blood glucose and glucose tolerance test of diabetic rabbits
[Values are mean±SD from 5 animals in each group]

Groups	Blood glucose (mg/dl)			Significance Group C Vs Group B
	Fasting	1 hr	2 hr	
Group A (healthy control)				
0 Week	80 ± 5	120 ± 8	78 ± 4	
2 week	82 ± 6	125 ± 5	80 ± 6	
Group B (diabetic)				
0 Week	140 ± 8	240 ± 10	160 ± 5	P>0.05
2 week	145 ± 6	260 ± 9	168 ± 7	
Group C (diabetic treated)				
0 Week	138 ± 5	270 ± 9	170 ± 7	
2 week	140 ± 7	280 ± 5	165 ± 9	

Hypercholesterolaemia can increase production of oxygen free radicals, which may result in lipid peroxidation leading to increased formation of malondialdehyde (MDA)[23,24]. Serum lipid peroxides were measured as the thiobarbituric acid reactive substances (TBARS). An increase in TBARS levels indicating decrease in antioxidant status in cholesterol fed animals observed in the present study. A decrease in the rise of TBARS after administration of OSSO suggests that this oil can decrease lipid peroxidation and improve antioxidant status. Decreased GSH levels in rabbits fed with cholesterol reflect increased oxidative stress due to cholesterol feeding. Treatment with OSSO along with cholesterol prevented increase in TBARS and the lowering of GSH. This is an indication that OSSO was able to reduce production of oxygen free radicals and thereby improve the antioxidant status.

While many reports are there on hypoglycaemic activity of OS leaves[1-3,8] there is only one report on OS seeds[3]. In the latter study, OS seed powder was given to normal rabbits for 4 weeks and 16% fall in FBG was observed. As seed powder showed hypoglycaemic effect, similar effect of OSSO was explored in the present study. However, no improvement in glucose tolerance or fall in FBG on two week treatment of diabetic rabbits with OSSO was observed.

In conclusion, results of the present study demonstrate that OSSO does not possess antidiabetic effect, but has significant hypolipidaemic and antioxidant activity. Lipid lowering effect may be due to some constituent in oil which either increase catabolism or interfere with absorption of cholesterol. The antioxidant effect of OSSO may be related to its hypocholesterolaemic property. Further studies are in progress to understand the mechanism of the lipid lowering effect of OSSO.

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