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Actions Underlying Antidiabetic Effects of *Ocimum sanctum* Leaf Extracts in Animal Models of Type 1 and Type 2 Diabetes

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JMAH, LA, BR, JK, MA, PRF and YHAAW designed the study. Authors JMAH, LA, BR, JK and MA conducted the experiment. Authors PRF and YHAAW supervised the study. Authors JMAH and OOO performed data analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study investigated mechanisms by which *O. sanctum* leaf extracts ameliorate hyperglycaemia using animal and cellular models of diabetes.
Place and Duration of Study: Diabetes Research Laboratory, University of Ulster, Coleraine, United Kingdom and Research Division, BIRDEM, Dhaka, Bangladesh; 8 months.
Methodology: Acute anti-diabetic effects of ethanolic extracts of *O. sanctum* were examined in normal and chemically-induced type 1 and 2 diabetic rats. Effects of extracts on glucose absorption, intestinal disaccharidase activity and gastrointestinal motility in type 2 diabetic rats and

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on glucose uptake and insulin action in 3T3-L1 cells were assessed.
Results: Treatment with the extract (1.25 g/kg bw) significantly improved oral glucose tolerance in normal and type 2 diabetic rats and suppressed blood glucose elevation after oral sucrose (2.5 g/kg bw) administration. The extract significantly reduced glucose absorption, gastrointestinal motility and disaccharidase activity. A 28-day treatment with *O. sanctum* decreased serum glucose, increased liver glycogen and enhanced circulating insulin and total oxidant status in type 2 diabetic rats. Glucose transport and insulin action in 3T3-L1 were increased by extract.
Conclusion: *O. sanctum* represents a useful as a source for discovery of novel antidiabetic compounds and as a dietary adjunct for the management of type 2 diabetes and its complications.

Keywords: Ocimum sanctum; 3T3 adipocytes; intestinal disaccharidase activity; hepatic glycogen; glucose uptake.

1. INTRODUCTION

Although, classical oral antihyperglycaemic agents are the mainstay treatment of type 2 diabetes, they fail to prevent diabetic complications [1]. This, therefore, justifies the search for more efficacious drugs. Although modern medicine has provided drugs belonging to classes including thiazolidinediones, GLP-1 mimetics and DPP-IV inhibitors [2], there is still a need for new agents with better potential and physiological antidiabetic actions for the treatment of diabetes. Therapeutic potential of phytochemicals in the management of many human diseases, including diabetes, have been widely acknowledged. However, scientific studies assessing the potentially important benefits of plants used traditionally for the treatment of diabetes are still limited [3,4].

Ocimum sanctum Linn (Labiatae), commonly known as 'Holy basil' is a herbaceous plant found throughout the Southern Asian region. It grows wild in India but is also widely cultivated for food in many homes and temple gardens due to its religious significance. *O. sanctum* has a long history of medicinal use and was mentioned in Charak Samhita, the ancient textbook of Ayurveda. The leaf of *O. sanctum* has been reported to contain bioactive phytochemicals such as saponin, flavonoids, triterpenoids, and tannins [5]. Specific bioactive compounds previously isolated from *O. sanctum* leaf are presented in (Table 1) [6-8]. The use of *O. sanctum* leaves in conditions, including catarrhal bronchitis, bronchial asthma, dysentery, dyspepsia, skin diseases, chronic fever, haemorrhage, helminthiasis and ring worms have been reported [9,10]. Fresh leaves of basil taken together with black pepper are used as a prophylactic treatment for malaria [11].

Extracts of *O. sanctum* leaves have been shown to exert hypoglycaemic effects in various experimental animals [12,13]. Ethanol extracts reduced blood glucose in normal, glucose-fed hyperglycaemic and streptozotocin-diabetic rats [12]. A diet containing *O. sanctum* leaf powder fed to diabetic rats for 1 month also significantly decreased fasting blood glucose [13]. Ethanol extracts of *O. sanctum* have also been reported to exhibit acute and chronic beneficial effects in alloxan-induced diabetes in rats [14]. Finally, in a randomized, placebo-controlled, clinical trial, leaf extract of *O. sanctum* caused a significant decrease in fasting and post-prandial glucose [15]. These observations plus the evidence that compounds isolated from ethanol extracts of *O. sanctum* exert antioxidant effects [6], illustrate the value of further studies to elucidate the antidiabetic actions of this plant.

We have reported that extracts of leaves of *O. sanctum* enhance insulin secretion in isolated perfused pancreas, isolated islets and clonal insulin secreting cells [16]. In the present study, we have investigated other possible mechanisms by which *O. sanctum* leaf extracts ameliorate hyperglycaemia using animal and cellular models of diabetes.

2. MATERIALS AND METHODS

2.1 Chemicals and Instrumentation

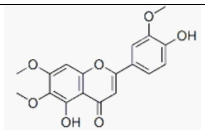
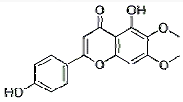
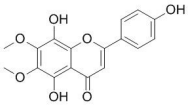
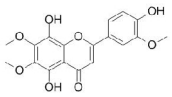
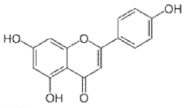
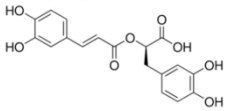
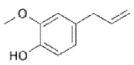
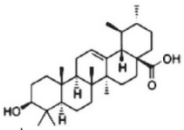
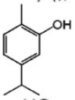
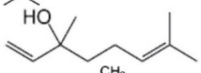
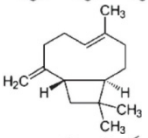
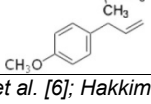
Reagents of analytical grades and deionized water (Purite, Oxon, UK) were used for the study. Steptozotocin and sodium pentobarbital were purchased from Sigma-Aldrich (St Louis, MO, USA). Sodium chloride, D-glucose, sucrose, ethanol, barium sulphate, carboxy methyl cellulose, calcium chloride, potassium chloride and sodium hydrogen carbonate were obtained from BDH Chemical Ltd (Poole, Dorset, UK). Rat insulin standard was obtained from Novo Industrial (Copenhagen, Denmark) and tritiated 2-deoxyglucose was supplied by Amersham International Plc (Burkinghamshire, UK). Hanks balanced salt solution; trypsin, RPMI-1640 tissue culture medium, foetal bovine serum and

penicillin/streptomycin were ontained from Gibco Life Technologies Ltd (Paisley, UK). The DC protein kit was procured from Bio-Rad, USA while all other kits were purchased from Boehringer Mannheim GmbH, Germany. Wallac 1409 scintillation counter was supplied by Wallac, Turke, Finland while the microwell plate ELISA reader was obtained from Bio-Tek, USA.

2.2 Plant Material Preparation

O. sanctum leaves were obtained from Ramkrishna Mission, India and voucher specimens were deposited in the National Herbarium, Bangladesh after botanical authentication. Leaves were dried at room temperature and pulverized prior to extraction of powdered samples (2 kg) with ethanol (80%, 10 l) for approximately 4 days at room temperature. The extraction solvent was changed daily and the combined extract was filtered, evaporated to dryness using a rotary evaporator and freeze dried (yield = 275 g, 13.8%). The sample was kept at 4ºC until used for assays.

Table 1. Active constituents isolated from *Ocimum sanctum* leaf

S/no	Common name	Chemical structure	IUPAC name
1	Cirsilineol		4',5-Dihydroxy-3',6,7-trimethoxyflavone
2	Cirsimaritin		5,4'dihydroxyl-6,7-dimethoxyflavone
3	Isothymusin		6,7-dimethoxy-5,8,4'-trihydroxyflavone
4	Isothymonin		4',5,8-Trihydroxy-3',6,7-trimethoxyflavone
5	Apigenin		5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one
6	Rosmarinic acid		(2*R*)-2-[[(2*E*)-3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]]oxy]-3-(3,4-dihydroxyphenyl)propanoic acid
7	Eugenol		4-Allyl-2-methoxyphenol
8	Urosolic acid		3-beta-3-hydroxy-urs-12-ene-28-oic-acid
9	Carvacrol		2-Methyl-5-(1-methylethyl)-phenol
10	Linalool		3,7-dimethylocta-1,6-dien-3-ol
11	Caryopylline		4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene
12	Estragol		1-methoxy-4-(2-propenyl)-benzene

Kelm et al. [6]; Hakkim et al. [7]; Rahman et al. [8]

2.3 Laboratory Animals and Induction of Diabetes

Male Long-Evans rats (14 weeks old, weighing 180 - 220 g) maintained on a standard rodent diet (containing 36.2% carbohydrate, 20.9% protein, 4.4% fat and 38.5% fibre with energy content of 282 Kcal/100 g) with 12 h light-dark cycle at 21±2ºC and water *ad libitum* were used in the present study. All animal experiments were carried out in accordance with local regulations and the UK Animals (Scientific Procedures) Act 1986 and EC Directive 86/609/EEC for animal experiments. Type 1 diabetes was induced in adult rats by a single i.p. injection of streptozotocin (STZ, 65 mg/kg bw) as previously described by Hannan et al. [22] while type 2 diabetes was induced by a single i.p. injection of STZ (90 mg/kg bw) 48h-old rats as previously described [17-19]. For type 1 diabetes, animals with blood glucose level >20 mM were included in the experiment. For type 2 diabetes, animals with clearly manifested features of obesity (body weight = 175 – 180 g), hyperglycaemia (glucose level >8mmol/l) and insulin resistance (adjudged by results of a prior insulin sensitivity test) were used for the experiments.

2.4 Oral Glucose Tolerance Tests

Acute glucose tolerance (oral, 32% w/v) tests were performed in overnight fasted non-diabetic and diabetic (type 1 and type 2, n=8) in the presence or absence of ethanol extract of *O. sanctum* (1.25 g/kg bw) as previously described [20]. For chronic studies, type 2 diabetic rats (n = 8) were treated with ethanol extracts of *O. sanctum* (1.25 g/kg body weight, twice-daily) or saline vehicle (0.9%, w/v, NaCl) over a 28-day period. Blood samples were collected at the times indicated in the (Fig. 1) for biochemical analysis as described previously [21,22]. Pancreas and liver were excised for determination of insulin content by ELISA [23] using commercial rat insulin kit from Crystal Chemical Inc., USA according to the manufacturer's protocol. Rat insulin standards were prepared over a concentration range of 0 – 2500 ρg/ml.

2.5 Sucrose Absorption in the Gut

Normal and type 2 diabetic rats were administered sucrose solution (2.5 g/kg body weight) alone or in combination with *O. sanctum* extract (2.5 g/kg body weight) after an overnight fast. Blood samples were collected by tail vein puncture at prior to sucrose administration and at times indicated in (Figs. 1G and 1H) for blood glucose measurement. Another set of rats were killed at intervals indicated in (Fig. 2) after sucrose administration and their gastrointestinal tract excised were divided into 6 segments as shown in the figure. Glucose liberated from each segment was measured as previously described [22,24]. Gastrointestinal sucrose content was calculated from the amount of glucose liberated as described by Goto et al. [24].

2.6 Intestinal Glucose Absorption

Effects of *O. sanctum* extracts (25 mg/ml) on intestinal glucose absorption were assessed by *In situ* intestinal perfusion as previously described [22] in non-diabetic rats. The perfusate was collected from a catheter set at the end of ileum at room temperature and a perfusion rate 0.5 ml/min for 30 min. Absorbed glucose was estimated from the concentration of glucose in the solution before and after the perfusion.

2.7 Intestinal Disaccharidase Activity and Gastrointestinal Motility

Intestinal disaccharidase activity in normal rats in the presence of ethanol extract of *O. sanctum* (1.25 g/kg) or vehicle was assessed as previously described [22]. Glucose and protein concentrations in the homogenate of small intestines excised from treated animals 1h after administration was measured as previously reported [22]. Gastrointestinal motility was evaluated using barium sulfate milk containing 10% w/v $BaSO_4$ in 0.5% carboxy methyl cellulose (CMC) [25]. The distance traversed by BaSO4 milk was measured and reported as a percentage of the total length of small intestine [22].

2.8 Measurement of Total Antioxidant Status (TAS)

Total antioxidant status was measured using serum of treated and control animals with 6-hydroxy-2, 5, 7, 8-tetramethylchriman-2-carboxylic acid as standard as described previously [22]. Following 3 min incubation of standard or serum samples (5µl) with the chromogen reagent (200µl, containing metmyoglobin and 2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) plus H_2O_2, absorbance was measured at 630nm. Unknown concentration of TAS was estimated from the standard curve.

2.9 Glucose Uptake and Insulin Action

Effects of *O. sanctum* extracts on glucose uptake and insulin action were examined using 3T3-L1 cells as reported earlier [20,22]. Glucose uptake was initiated by the addition of tritiated 2-deoxyglucose (0.5 μCi/well, 50 μl) at glucose concentration of 50mM. Radioactivity was measured on a Wallac 1409 scintillation counter (Wallac, Turke, Finland) and result expressed as disintegration per min (DPM) [26].

2.10 Statistical Analysis

Results are expressed as the mean ± SD. Values were compared by Student's unpaired *t*-test and Mann-Whitney U test where appropriate. Data at different time points were analysed by one way ANOVA with Bonferroni adjustment. Pair-wise comparisons to the control group were performed using Dunnett's test. Groups of data were considered to be significantly different if $P<0.05$. Analyses were carried out using GraphPad Prism (GraphPad Software, Inc., USA).

3. RESULTS

3.1 Acute and Long-term Effects of Ethanol Extract of *O. sanctum* on Glucose Tolerance

Fasting serum glucose concentrations in normal and type 2 diabetic rats were reduced significantly following oral administration of ethanol extracts of *O. sanctum* (Fig. 1). Co-administration of glucose and the extracts also produced a significant improvement in glucose tolerance in normal and type 2 diabetic rats. Significantly lower serum glucose levels were observed at 30min ($p<0.01$ normal, $p<0.001$ type 2 diabetic) and 75 min ($p<0.05$) in both groups of rats (Figs. 1A, C). The extracts produced no anti-hyperglycaemic effect in rats with type 1 diabetes (Fig. 1B).

Twice-daily administration of *O. sanctum* extract for 28 days resulted in reduced serum glucose by 29% ($p<0.01$) and elevated serum insulin by 41% ($p<0.001$) in rats with type 2 diabetes compared with their saline controls. While pancreatic insulin content remain unchanged, hepatic glycogen concentration and total antioxidant status (TAS) were increased by 1.7-fold and 1.4-fold respectively after the administration of *O. sanctum* extract to type 2 diabetic rats (Table 2).

3.2 Effects of *O. sanctum* on Sucrose Tolerance and Absorption in the Gut

A sharp rise followed by a gradual release in blood glucose concentration was observed at 30 min post sucrose administration in normal and type 2 diabetic control rats (Figs. 1G and 1H). This rapid increase was significantly inhibited in both normal and type 2 diabetic rats treated with ethanol extracts of *O. sanctum* with marked differences observed at 30 min ($p<0.05$) and 60 min ($p<0.01$) post sucrose administration. Following a 20 h fast and a sucrose load of 425 mg/rat to non-diabetic control rats, significantly high concentration of sucrose was detected in the stomach and the upper and middle small intestine within 30 min of sucrose administration (Figs. 2A –C). Sucrose concentration in all these region were rapidly reduced within 2h post sucrose administration except in lower small intestine where an increase was observed at 1h post sucrose administration (Fig. 2D). Co-administration of sucrose and *O. sanctum* extract did not affect sucrose concentration at 30min in the stomach and lower part of small intestine but produced a slower rate of absorption of sucrose across the small intestine (Fig. 2A-D). Compared with non-diabetic controls, significantly ($p<0.05$) higher concentration of sucrose was observed all the different regions of the GI tract in non-diabetic mice administered *O. sanctum* extracts with sucrose. Compared to other parts of the GI tract, significantly lower concentration of sucrose was observed in the caecum and larger intestine. Higher inhibition of sucrose absorption was observed in the caecum of non-diabetic rats administered *O. sanctum* while a relatively similar pattern of absorption was observed in the large intestine of these rats and control rats (Figs. 2E-F). In type 2 diabetic control rats, a more rapid rate of sucrose clearance was observed compared with their non-diabetic counterparts. Lower concentration of sucrose was observed, particularly in the caecum of type 2 diabetic control rats compared with non-diabetic rats (Fig. 2). Administration of *O. sanctum* extracts significantly inhibited sucrose absorption in type 2 diabetic rats with significant accumulation observed in all the regions of small intestine and caecum ($p<0.05$, Figs. 2H – K) for up to 2h post sucrose administration. Sucrose absorption in the large intestine of both groups of type 2 diabetic rats were not significant different except at time point 2h post injection where sucrose accumulation was observed in rats treated with *O. sanctum* extracts ($p<0.05$, Fig. 2L).

Table 2. Effects of ethanol extract of *Ocimum sanctum* leaves on serum levels of glucose and other parameters in type 2 diabetic rats after 28 days of feeding

Parameters	Day 0		Day 28	
	Control	*O sanctum*	Control	*O sanctum*
Glucose (mmol/l)	8.84±1.3	8.76±1.2	8.90±1.02	6.18±0.9**
Insulin (ng/ml)	0.434±0.1	0.446±0.2	0.442±0.1	0.628±0.1*
Pancreatic insulin (ng/g pancreas)			0.88±0.34	0.96±0.61
Liver glycogen (g/100g tissue)			0.79±0.2	1.34±0.4*
Total antioxidant status (mmol/l)	0.87±0.1	0.92±0.2	0.89±0.1	1.23±0.2**

*Diabetes was induced by injection of neonatal rats with 90 mg/kg STZ approximately three months previously. Dose of O sanctum was administered orally by gavage (1.25g/kg body weight) twice daily for 28 days. Data are presented as mean ± SD (n=12). Significance differences were compared by unpaired t test; *P<0.05, **P<0.01 compared to type 2 diabetic control rats*

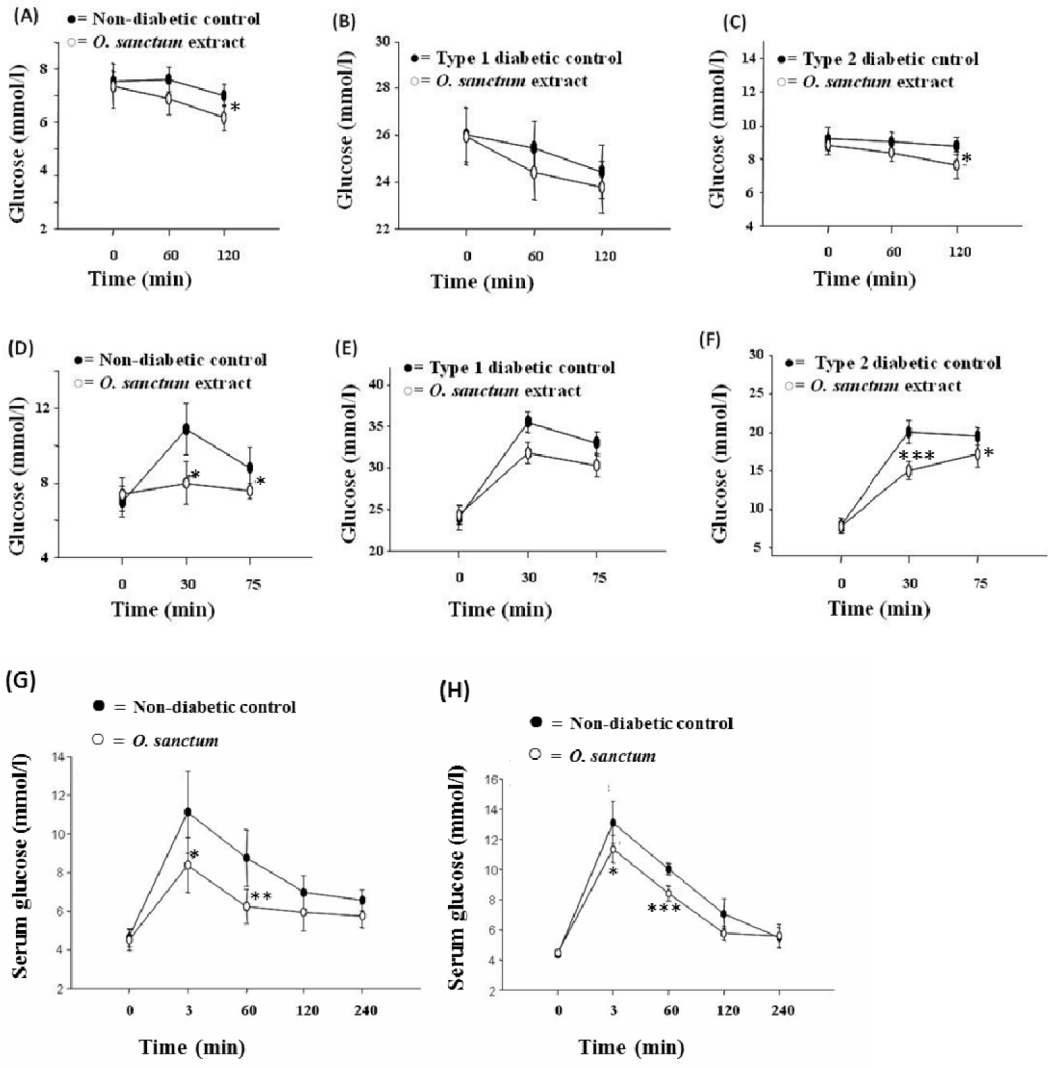


Fig. 1. Effects of ethanol extract of *O. sanctum* on fasting glucose (A – C) and glucose tolerance (D – F) in non-diabetic, type 1 and type 2 diabetic rats as well as serum glucose after sucrose load in non-diabetic (G) and type 2 diabetic rats (H)

*Results are mean ± SD depicted by vertical bars (n = 8). Fasted rats were given ethanol extract by gavage (1.25 g/kg body weight) with or without glucose (2.5g/kg body weight). For (G) and (H), rats were fasted for 20 h and administered sucrose solution (2.5 g/kg body weight) by gavage with or without ethanol extract of O. sanctum (1.25 g/kg body weight). Significances are derived from repeated measures ANOVA and adjusted using Bonferroni correction; *P<0.05, **P<0.01, ***P<0.001 compared to respective control rats*

3.3 Effects of *O. sanctum* Extracts on Intestinal Glucose Absorption

Intestinal glucose absorption was almost constant during 30 min of in situ perfusion with glucose. However, 13 - 19% reduction in the percentage glucose absorption was observed in the presence of *O. sanctum* extract during whole perfusion period ($p<0.05$, Fig. 3A).

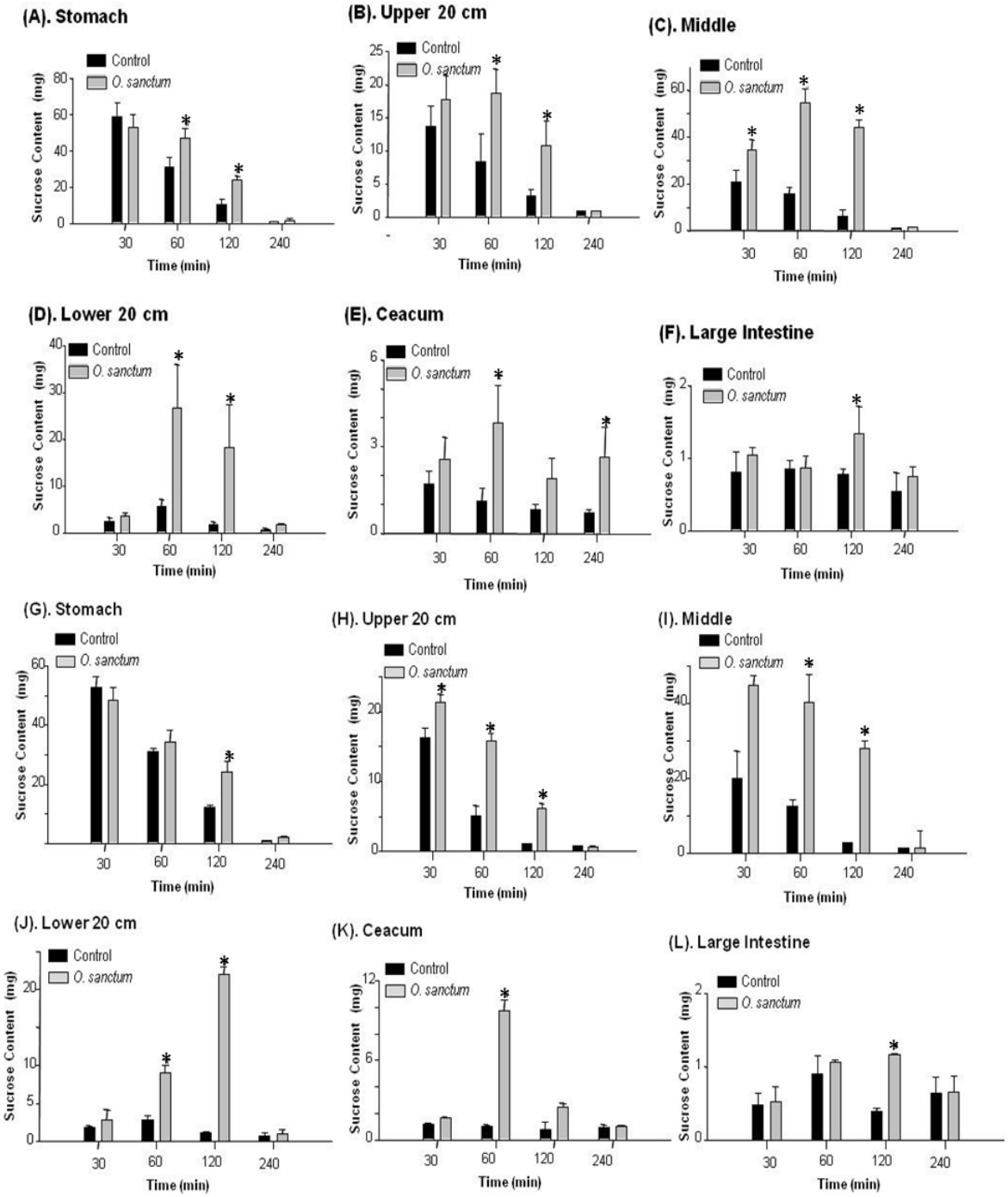


Fig. 2. Effects of ethanol extract of *O. sanctum* on gastrointestinal sucrose content after oral sucrose loading in non-diabetic (A – F) and type 2 diabetic rats (G – L)

*Rats were fasted for 20 h prior to administration of a sucrose solution (2.5 g/kg body weight) by gavage with or without ethanol extract (1.25 g/kg body weight). Results are mean ± SD (n = 8); *P<0.05 compared to control*

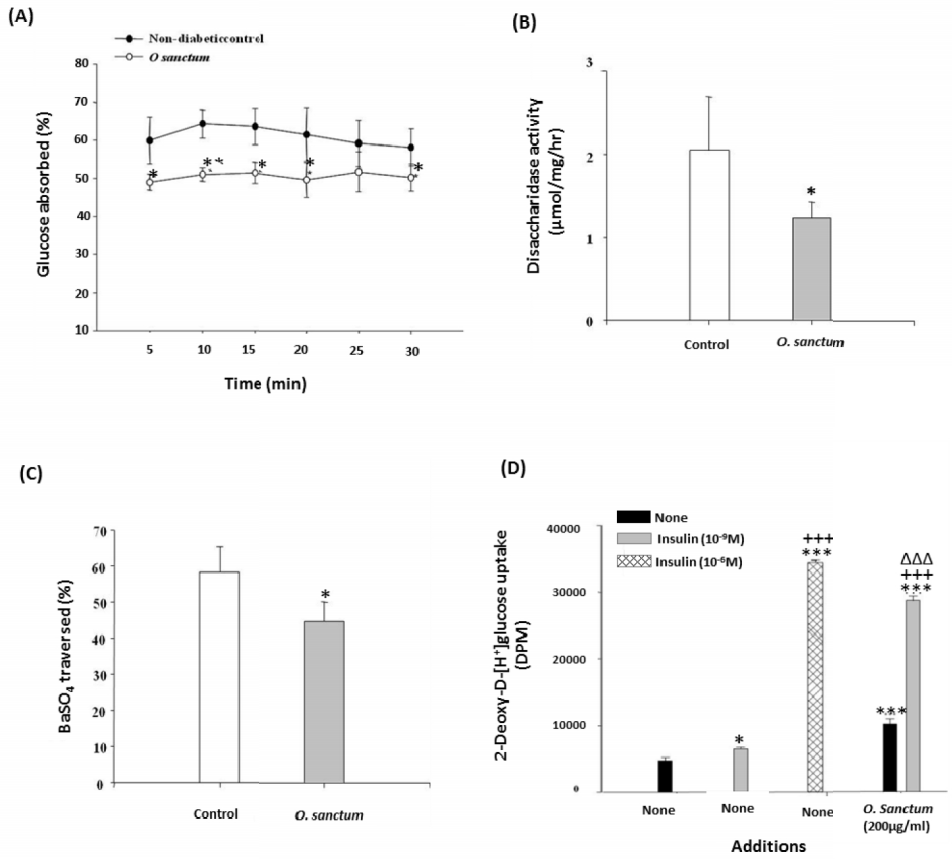


Fig. 3. Effects of ethanol extract of *O. sanctum* on intestinal glucose absorption (3A), intestinal disaccharidase activity (3B) and gastrointestinal motility (3C) in non-diabetic rats as well as glucose uptake by 3T3-L1 adipocytes (3D)

*For (A), rats were fasted for 36 h prior to glucose (54 g/l) perfusion with or without extract of O. sanctum (25 mg/ml) while rats were fasted for 20 h prior to administration of plant extract by gavage (1.25g/kg body weight) for 3B and 3C. Enzyme activity determination and $BaSO_4$ administration was at 60 min (3B). Motility was measured over the following 15 min (3C). Values are mean ± SD (n = 8). Significances are derived from repeated measures ANOVA and adjusted using a Bonferroni correction. *P<0.05, **P<0.01 compared to non-diabetic control (3A-C). For 3D, *P<0.05, ***P<0.001 compared to no insulin incubation, $^{\Delta\Delta\Delta}$P<0.001 compared to plant ethanol extract incubation without insulin and $^{+++}$P<0.0001 compared to 10^{-9}M insulin alone*

3.4 Effects of *O. sanctum* on Intestinal Disaccharidase Activity and Gastrointestinal Motility

Intestinal disaccharidase activity in non-diabetic rats was significantly inhibited (p<0.05) in the presence of the plant extract (Fig. 3B) and gastric mobility, measured by the percentage of intestinal length traversed by barium milk, decreased by 23% in rats treated with *O. sanctum* extracts compared to control rats (p<0.05, Fig. 3C).

3.5 Effects of *O. sanctum* on Glucose Uptake in 3T3 Adipocytes

The ethanol extract of *O. sanctum* significantly enhanced glucose transport compared with control incubations in the absence of insulin (p<0.001, Fig. 3D). The positive effects of *O. sanctum* extract were further increased by the presence of insulin (10^{-9} M, p<0.001, Fig. 3D).

4. DISCUSSION

The use of *O. sanctum* leaves in traditional management of diabetes has been widely reported and the traditional claims of the antihyperglycaemic effects of *O. sanctum* leaf extracts have been verified by a number of scientific studies [12,13,15]. To further understand the mechanisms of actions and therapeutic efficacy of *O. sanctum* extracts in the management of diabetes, our study examined acute and chronic antidiabetic actions of *O. sanctum* using non-diabetic and diabetic

animal models. We also uniquely investigated the extrapancreatic actions of *O. sanctum* and studied its effects on gastrointestinal tract motility, intestinal carbohydrate digestion and absorption, liver glycogen content and cellular glucose uptake.

Oral administration of ethanol extracts of *O. sanctum* leaves prevented elevated levels of fasting blood glucose and resulted in significant improvement of glucose tolerance in non-diabetic and type 2 diabetic rats with no beneficial effects observed in type 1 diabetic rats. Moreover, enhanced insulin secretion was observed in type 2 diabetic rats treated with *O. sanctum* extracts for 28 days. These observations and our earlier report of the insulinotropic effects of *O. sanctum* extracts on perfused pancreas, isolated islets and clonal pancreatic cells [16,27] suggest that the glucose lowering action is mediated partly through the stimulation of insulin release. Moreover, the lack of antihyperglycaemic effects in type 1 diabetic rats characterized by beta cell destruction further corroborates the assertion that *O. sanctum* extracts act, at least in part, by improving insulin secretion. Insulinotropic actions observed for *O. sanctum* extracts is consistent with observations reported for extracts of other plants, such as *Agrimony eupatoria* [28], *Terminalia bellirica* [29], *Asparagus adscendens* [4], *Sambucus nigra* [30], *Coriadrum sativum* [31], *Viscum album* [32] and many others [33] with insulin-releasing actions.

Consistent with the growing body of evidence showing that leaves of *O. sanctum* contain natural agents with reproducible antidiabetic effects, acute *In vivo* experiments conducted in this study revealed that oral administration of ethanol extracts of *O. sanctum* inhibited increased serum glucose level following an oral glucose load. This observation raises the possibility that *O. sanctum* may also act by interfering with intestinal glucose absorption in the postprandial state. Our previous studies with seed extracts of *Trigonella foenum-graecum* [34], root extracts of *Asparagus racemosus* [22] and husk extracts of *Plantago ovata* [20] produced a similar inhibition of intestinal glucose absorption as observed in this study.

It is known that improved glucose tolerance could be achieved in people suffering from diabetes by either slowing down gastric emptying, increasing intestinal nutrient transit or modification of the release or actions of key digestive enzymes [35]. It has also been shown that high-fibre diets and some plant products could help achieve improved tolerance through one or a combination of these approaches. We examined the effects of *O. sanctum* extract on sucrose digestion and absorption in the gut of normal and type 2 diabetic rats. Significant inhibition of postprandial hyperglycaemia and increased concentration of residual sucrose was observed, suggesting that the carbohydrate digestion or absorption was inhibited by the extract. While this result is consistent with previous findings [16], low levels of sucrose detected in the large intestines suggests that *O. sanctum* extracts may act by slowing down but not totally preventing sucrose absorption.

The hypothesis that antihyperglycaemic effects of the extract may be, at least partly, mediated through the retardation of carbohydrate digestion and absorption was further investigated by gut perfusion experiments, which showed that *O. sanctum* extracts significantly reduced intestinal glucose absorption. Consistent with other studies, intestinal disaccharidase activity was also significantly, suggesting that the reduction of absorption may also partly related to inhibition of intestinal disaccharidase activity [16,35]. Agents that inhibit carbohydrate digestion, such as α-glucosidase inhibitors, are currently in use for diabetes management [36]. However, it is not yet clear if the active principle in the ethanol extract of *O. sanctum* can be considered as a member of this group of agents. As indicated by experiments in non-diabetic rats, reduced gastric motility in the presence of *O. sanctum* extract may also contribute to the reduced rate of carbohydrate digestion and absorption observed in this study. While further investigations may be required to evaluate such effects in diabetic animal models, agents such as exanatide [37] and pramlinitide [38] that act partly by reducing gastric emptying are currently in use for diabetes management.

Impaired glucose uptake and utilization by peripheral tissues is one of the major derangements of type 2 diabetes [39]. We evaluated the effects of *O. sanctum* leaf extract on glucose uptake in the absence and presence of insulin using 3T3 adipocytes; a cell line that has been successfully used in previous studies to investigate effects of novel agents on glucose uptake [16,22]. Results revealed that *O. sanctum* extracts significantly enhanced glucose transport and that its effects were augmented in the presence of insulin. While further investigations are necessary to understand *In vivo* molecular

mechanisms through which *O. sanctum* extracts facilitate glucose uptake, its lack of effect in animal models of type 1 diabetes makes this effect of *O. sanctum* extracts less exciting.

Longer term *In vivo* studies were performed in rats with type 2 diabetes induced by a single intraperitoneal injection of streptozotocin (STZ) at 48 hours of age [40]. Increased total antioxidant status observed in rats treated with *O. sanctum* extracts for 28 days (Table 2) suggests that the extract may afford protection to pancreatic islets [41] against free radical mediated pancreatic β cells damage [42]. In addition, this action of the extract may be beneficial in preventing oxidative stress mediated diabetic complications [43]. Though the pancreatic insulin contents of control and *O. sanctum* treated rats were similar, increased hepatic glycogen content was observed in the group treated with the plant extract. Further investigations are necessary to examine if there is link between the actions of *O. sanctum* extracts on insulin-release, glucose uptake and hepatic glycogen deposition in diabetic rats [44].

5. CONCLUSION

Ethanol extracts of *O. sanctum* leaves improved glucose homeostasis in type 2 diabetes rats by enhancing circulating insulin and delaying carbohydrate digestion. *O. sanctum* therefore represents a useful as a source for discovery of novel antidiabetic compounds and as a dietary adjunct for the management of type 2 diabetes and its complications.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as the UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63EU for animal experiments. All experiments were examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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