ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECTS OF METHANOLIC EXTRACT OF CITRULLUS LANATUS SEEDS IN RATS

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ABSTRACT

Objective: Aim of the present study is to evaluate the antidiabetic and antihyperlipidemic effect of methanolic extract of Citrullus lanatus seeds (MECL) in streptozotocin (STZ) induced diabetic rats.

Methods: Diabetes was induced by a single dose of STZ (65 mg/kg) in citrate buffer, while the normal control group was given the vehicle only. After three days of induction of diabetes, the diabetic animals were treated further four weeks with MECL (200, 400 and 600 mg/kg) and glibenclamide (4 mg/kg). Blood glucose level was estimated every week of the study starting from d 1. At the end of study period, biochemical estimations of blood i.e. lipid profile was performed and animals were sacrificed to carry out liver glycogen estimations.

Results: At the end of study period, i.e. 28 d, STZ-induced rats showed marked hyperglycemia, hypertriglyceridemia and hypercholesterolemia. Body weight was reduced and the blood sugar level was significantly elevated in diabetic rats. The four-week treatment with MECL in a concentration of 200, 400 and 600 mg/kg significantly reversed the elevated levels of fasting blood glucose, serum cholesterol, serum triglyceride, liver glycogen, glycosylated hemoglobin with increase in body weight of diabetic rats.

Conclusion: Thus the present study suggested the useful potential of Citrullus lanatus seed in diabetes due to its antidiabetic, hypoglycemic and antihyperlipidemic properties.

Keywords: Streptozotocin, Citrullus lanatus, Cucurbitaceae.

INTRODUCTION

Diabetes mellitus (often abbreviated as DM) is the commonest endocrine and metabolic disorder that affects more than 100 million people in the world (6% population). It is caused by deficiency and/or ineffective insulin production by the pancreas which causes an increase in concentrations of glucose in the blood. It is found to damage many of body systems particularly eyes, blood vessels, kidney, nerves, heart and alters carbohydrate, lipid and protein metabolism in body [1]. Conventionally, diabetes mellitus has been divided into two broad types i.e. insulin dependent diabetes mellitus (IDDM, which is often known as Type I) and non-insulin dependent diabetes mellitus (NIDDM, known also as Type II). Type I diabetes is characterized by selective destruction of insulin secreting cells probably due to local inflammatory reactions in and around an islets in pancreas whereas Type II diabetes is characterized by either impaired insulin secretion or peripheral insulin resistance or both [2]. The people with DM also have increased risk of many complications such as peripheral vascular diseases, blindness, retinopathy, cardiovascular diseases, neuropathy, stroke, renal failure, gangrene, infections, obesity, amputations etc [3]. Drugs such as insulin or oral hypoglycemic agents such as biguanid es or failure, gangrene, infections, obesity, amputations etc [3]. Drugs such as insulin or oral hypoglycemic agents such as biguanid es or failure, gangrene, infections, obesity, amputations etc [3]. Drugs such as insulin or oral hypoglycemic agents such as biguanid es or failure, gangrene, infections, obesity, amputations etc [3]. Drugs such as insulin or oral hypoglycemic agents such as biguanid es or failure, gangrene, infections, obesity, amputations etc [3]. Drugs such as insulin or oral hypoglycemic agents such as biguanid es or failure, gangrene, infections, obesity, amputations etc [3]. Drugs such as insulin or oral hypoglycemic agents such as biguanid es or failure, gangrene, infections, obesity, amputations etc [3].

Various plants showed hypoglycemic effect due to presence of phyto constituents present in them. These phyto constituents are acting by increasing insulin secretion from beta cells in pancreas [7]. Cucurbitaceae plants contain bioactive compounds such as triterpenes, sterols, cucurbitacin and alkaloids. In the Middle East and Asia such plants were used effectively and extensively as herbal remedies for the variety of ailments. Citrullus lanatus (known as watermelon) is used widely in traditional system of Ayurveda. The fruit is diuretic and effective in the treatment of renal stones and dropsy. The rind of the watermelon fruit is also prescribed in cases of alcoholic poisoning and diabetes [7]. The root is laxative and in large doses it is emetic. The seed is diuretic, demulcent and tonic. It is sometimes used to treat urinary infection, or to clear urinary passage and to reduce bed wetting. The seed is also a used to expel parasitic worms (vermifuge) as it contains fatty oils in seed or its aqueous or alcoholic extracts are used to paralyze tapeworms and roundworms. It is also having blood pressure lowering property. In Northern Sudan, it is often used for treating burns, swelling and rheumatoid arthritis and also used as purgative [7]. Previous researches have reported that, leaves, flowers and fruits of watermelon contain vitamins, vital nutrients, fibers, phytochemicals, amino acids, fats, minerals and antioxidants. It is also shown that the consumption of at least 5-10 serving daily significantly reduces the risk of chronic diseases and supplies adequate amount of nutrients for maintaining good health [8]. It also contains terpenoids, steroids, flavonoids, tannins, carotenoids, alkaloids and glycosides which all act as antibiotics. These phytochemicals have been studied for their antifungal, anti-inflammatory and fungistatic activities [9]. Although leaves, flowers, stem, root and whole plant were studied in many researches for treatment of various disorders due to presence of useful phytochemicals, a very few reports are available on seeds as a source of phytoconstituents and their useful benefits. The seeds are often treated and ignored as waste part of plant, being a good source of phytoconstituents. The seeds contain flavonoids and these flavonoids reduce blood sugar by stimulating insulin release from pancreas [10]. But none of the reports are available to show beneficial effect of flavonoids present in seed in diabetes mellitus. This paper is...
therefore an effort to screen antidiabetic and antihyperlipidemic potential of phytoconstituents present in watermelon seeds.

**MATERIALS AND METHODS**

**Collection and authentication of Citrullus lanatus**

Fresh seeds of watermelon were purchased from local markets of Pune and authenticated by J. Jayanthi at Botanical Survey of India, Koregaon Road, Pune. They were washed, dried and crushed to make the coarse powder. (Voucher No. BSI/WRC/Tech/2012).

**Preparation of extract**

The crushed material was weighed exactly about 650 g and ground using mixer grinder to obtain the fine powder. It was then extracted with 80% methanol by Soxhlet extractor for 7 d in the dark under room temperature with intermittent shaking. After 7 d, the extracted material was collected and filtered using the muslin cloth at first and then through filter paper. It was evaporated to dryness under low pressure and temperature to get the concentrated extract. It was then preserved first in a desiccator for 3 d and then transferred to the refrigerator to carry pharmacological studies [11].

**Chemicals**

Streptozotocin (STZ) and Glibenclamide (GI) were purchased from New Neeta Chemicals, Pune (Bill No.1486). Glucose, Triglyceride, Total cholesterol and HDL cholesterol kits were purchased from Universal Diagnostics, Pune (Bill No. 256). All other chemicals and glasswares were arranged from the central store of MAEER'S Maharashtra Institute of Pharmacy, Pune. Accu-check glucometer with 100 strips was purchased online from Bantom Laboratories Pvt Ltd, Paharganj, New Delhi (Invoice no. C29131/14-15/0224).

**Animals**

Male Wistar rats of seven weeks old (150-200 g) were obtained from New Institute of Bioscience, Sinhgad Road, Pune (Invoice no. B-30). Before and during experimental rats were fed with standard pellet diet (Nutrivit life sciences, Pune) with easy and free access to water. They were allowed to acclimatize for a period of 7 d under the standard environmental condition of relative humidity, temperature and light/dark cycle. After 7 d, they were weighed and divided into various groups. Animals stated as fasting were deprived of food and water for 12 h ad libitum. All the experimental procedures were carried out were as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA). The study was reviewed and approved by the Institutional Animal Ethical Committee (IAEC), Maharashtra Institute of Pharmacy, Kothrud, Pune, (Approval No. MIP/IAEC/2013-2014/M1/Apr/001).

**Acute and short-term toxicity study**

The methanolic extract of Citrullus lanatus (MECL) seeds was tested for its acute and short-term toxicity in rats. To determine acute toxicity of the drug, overnight fasted Wistar rats were orally fed with extract in increasing dose as 100, 300, 500, 1000, 1500 and 5000 mg/kg body weight. The general behavior and mortality of animals were observed continuously for an initial period of 2 h, 4 h, 8 h, 12 h then again at 24 h, 48 h and 72 h following drug administration. The parameters observed with the higher dose more than 1300 mg/kg were loss of reflex, grooming, sedation etc. There was no death of animal [6, 12].

**Determination of test dose**

During preliminary toxicity study, there was no any mortality observed in experimental animals with oral administration of seed extract up-to the high dose of even 5 g/kg body weight for 48 h. Hence, sub-maximal doses of 200, 400 and 600 mg/kg were selected as the test dose.

**Experimental induction of diabetes**

Diabetes was induced by diabetogenic agent, streptozotocin (STZ). The solution was injected once intra-peritoneally in a dose of 65 mg/kg in rats. 5% glucose solutions were fed orally for 24 h to prevent any death due to initial hypoglycemia induced by streptozotocin. After 72 h of STZ injection, fasting blood glucose levels were tested using glucose oxidase-peroxidase reactive strips with Accu-Chek glucometer. Rats showing fasting blood glucose level more than 200 mg/dl were considered diabetic and used for further study [6, 13].

**Experimental animal groups**

Rats were divided into different groups as follows

- Group I-as normal control where rats received citrate buffer daily.
- Group II-as diabetic control where rats received citrate buffer daily and STZ in a dose of 65 mg/kg once on first day (diabetic induced group).
- Group III-diabetic rats receiving 200 mg/kg methanolic extract of seeds of Citrullus lanatus.
- Group V-diabetic rats receiving 400 mg/kg methanolic extract of seeds of Citrullus lanatus.
- Group VI-diabetic rats receiving 4 mg/kg Glibenclamide as the oral hypoglycemic agent (standard drug group).

**Experimental procedure**

**Blood glucose estimation**

To check the blood glucose levels, blood was obtained from retro-orbital sinus of anesthetized rats with the help of capillary tube. Before induction of diabetes, fasting blood glucose of all rats was determined to know normal blood glucose level. After diabetes induction, fasting blood glucose in all experimental rats was determined initially for 3 d, to check diabetic status of animals and thereafter every week during the 28 d study period. Blood glucose levels were determined using Accu-Chek Glucometer.

**Serum lipid profile estimation**

At the end of 28 d, blood was collected from retro-orbital sinus of anesthetized rats and serum was separated to determine parameters like total cholesterol, HDL cholesterol, ratio of total cholesterol and HDL, VLDL, LDL and triglycerides using commercially available kits (Universal diagnostics, Guruwar Peth, Pune). Some parameters were tested in the pathology lab (Joshi lab, Sadashiv Peth, Pune).

**Glycosylated hemoglobin (HbA1c)**

At the end of 28 d, blood was collected and transferred to pathology lab (Joshi lab, Sadashiv Peth, Pune) for the determination of glycosylated hemoglobin.

**Liver glycogen estimation**

At the end of 28 d, animals were sacrificed by cervical dislocation and dissected to isolate liver of an individual animal and it was homogenized in 5% w/v trichloroacetic acid and its glycogen content was determined by the method of Carrol [14].

**Body weight**

Initial (0 d) and final (28 d) body weight was measured on digital weighing balance.

**Statistical analysis**

All results are expressed as the mean±SEM. The results were analyzed for statistical significance by one-way ANOVA followed by Dunnets's Multiple Test of Comparison.

**RESULTS**

The effect of STZ and methanolic extract Citrullus lanatus seed (MECL) on fasting blood glucose level is shown in table 1. On repeated administration of MECL for 28 d, significant (p<0.01) and sustained decrease in the blood glucose level of diabetic rats was observed in a dose-dependent manner as compared with the diabetic control group. In diabetic rats, blood glucose level was reduced by 57.9 mg%, 66.4 mg% and 93 mg% in 200, 400 and 600 mg/kg doses of MECL respectively (28 d). The standard oral hypoglycemic drug glibenclamide (4 mg/kg) showed more potent antidiabetic activity by reducing blood glucose level by 108.2 mg% as compared with a diabetic control group (28 d).
As shown in table 2, STZ diabetic rats treated with MECL showed significant \( (p<0.01) \) reduction in the elevated level of total cholesterol \((T-CH)\) and triglycerides \((TG)\) as compared to diabetic rats. Chronic treatment of extract \( (600 \text{ mg/kg}) \) and glibenclamide \( (4 \text{ mg/kg}) \) reduced total cholesterol by \(28.7\%\) and \(41.5\%\) respectively. Similarly extract \( 600 \text{ mg/kg} \) reduced low density lipoprotein \((LDL)\), very low-density lipoprotein \((VLDL)\) and triglycerides by \(19.01\%, 6.99\% \) and \(20.2\%\) respectively whereas glibenclamide reduced it by \(20.99\%, 8.74\%\) and \(11.7\%\) respectively. Also the extract significantly \( (p<0.01) \) improved the high-density lipoprotein \((HDL)\) level at \(600 \text{ mg/kg} \) dose. In addition, the extract in a dose of \(600 \text{ mg/kg} \) showed a significant reduction in \(T-CH\) and \(HDL\) ratio.

As shown in table 3, there was a significant increase in blood glycosylated hemoglobin and decrease in liver glycoce levels in STZ diabetic rats as compared to normal rats. Oral administration of the extract \((600 \text{ mg/kg}) \) significantly \( (P<0.01) \) restored the increased glycosylated hemoglobin and decreased liver glycoce level in diabetic rats as glibenclamide.

As shown in table 4, STZ diabetic rats showed significant \( (p<0.01) \) reduction in body weight from \(238.2 \text{ g} \) to \(127 \text{ g} \) as compared with the normal group. Oral administration of MECL \((600 \text{ mg/kg}) \) significantly \( (P<0.01) \) and periodically improved the body weight after \(28 \text{ d} \) as compared to diabetic control.

### Table 1: Effect of chronic administration \((28 \text{ d})\) of MECL on fasting blood glucose in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Fasting blood glucose (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 0</td>
</tr>
<tr>
<td>Normal control</td>
<td>112.5±7.50</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>434.5±9.47</td>
</tr>
<tr>
<td>Diabetic+200 mg/kg MECL</td>
<td>429.5±8.04</td>
</tr>
<tr>
<td>Diabetic+400 mg/kg MECL</td>
<td>423.7±8.45</td>
</tr>
<tr>
<td>Diabetic+600 mg/kg MECL</td>
<td>402.0±20.81</td>
</tr>
<tr>
<td>Diabetic+4 mg/kg GL</td>
<td>417.7±14.47</td>
</tr>
</tbody>
</table>

\(n=6, *p<0.05, **p<0.01\). Values are mean±SEM. MECL: Methanolic extract of Citrullus lanatus seeds; GL: Glibenclamide. Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet’s multiple test of comparison.

### Table 2: Effect of chronic administration \((28 \text{ d})\) of MECL on lipid profile in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Lipid profile (mg %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-CH</td>
</tr>
<tr>
<td>Normal control</td>
<td>90.13±3.33</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>147.3±16.33</td>
</tr>
<tr>
<td>Diabetic+200 mg/kg MECL</td>
<td>134.6±13.08''</td>
</tr>
<tr>
<td>Diabetic+400 mg/kg MECL</td>
<td>127.9±8.76''</td>
</tr>
<tr>
<td>Diabetic+600 mg/kg MECL</td>
<td>118.6±18.15''</td>
</tr>
<tr>
<td>Diabetic+4 mg/kg GL</td>
<td>105.8±8.15</td>
</tr>
</tbody>
</table>

\(n=6, *p<0.01, \ ns: \text{non-significant. Values are mean±SEM, MECL: Methanolic extract of Citrullus lanatus seeds; GL: Glibenclamide. Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet’s multiple test of comparison.}

### Table 3: Effect of chronic administration \((28 \text{ d})\) of MECL on liver glycogen and glycosylated hemoglobin in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Liver glycogen (g/100 g)</th>
<th>Glycosylated hemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.67±0.18</td>
<td>5.3±0.41</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.34±0.02</td>
<td>6.5±0.43</td>
</tr>
<tr>
<td>Diabetic+200 mg/kg MECL</td>
<td>1.18±0.06</td>
<td>5.9±0.43</td>
</tr>
<tr>
<td>Diabetic+400 mg/kg MECL</td>
<td>1.91±0.07</td>
<td>5.7±0.34</td>
</tr>
<tr>
<td>Diabetic+600 mg/kg MECL</td>
<td>2.35±0.11</td>
<td>5.3±0.37</td>
</tr>
<tr>
<td>Diabetic+4 mg/kg GL</td>
<td>3.07±0.07</td>
<td>5.1±0.30</td>
</tr>
</tbody>
</table>

\(n=6, *p<0.01. \text{Values are mean±SEM, MECL: Methanolic extract of Citrullus lanatus seeds; GL: Glibenclamide, Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet’s multiple test of comparison.}

### Table 4: Effect of chronic administration \((28 \text{ d})\) of MECL on body weight in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Normal control</td>
<td>246.2±13.45</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>239.2±7.44</td>
</tr>
<tr>
<td>Diabetic+200 mg/kg MECL</td>
<td>239.9±7.65</td>
</tr>
<tr>
<td>Diabetic+400 mg/kg MECL</td>
<td>241.2±7.13**</td>
</tr>
<tr>
<td>Diabetic+600 mg/kg MECL</td>
<td>243.8±6.41</td>
</tr>
<tr>
<td>Diabetic+4 mg/kg GL</td>
<td>236.9±5.91</td>
</tr>
</tbody>
</table>

\(n=6, *p<0.01, \ ns: \text{non-significant. Values are mean±SEM, MECL: Methanolic extract of Citrullus lanatus seeds; GL: Glibenclamide, Data analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnet’s multiple test of comparison.}

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DISCUSSION

The occurrence and progression of type 2 diabetes is related with pancreatic β-cell dysfunction or damage which occurs together with or without insulin resistance. Normal β-cell can overcome insulin resistance by secretion of insulin level, but insufficient outcome leads to the onset of glucose intolerance. Once hyperglycemia becomes prominent, pancreatic β-cell function gradually start impairing, leading to further impairment of glucose-induced insulin secretion. The cause of bioavailability of β-cell occurs often accompanied by a decrease in a number of β-cell. Hyperglycemia is a direct cause of this effect. Chronic hyperglycemia may completely impair β-cell function at the level of insulin synthesis as well as insulin release [15]. If this diabetes remain untreated for longer period, then it ultimately leads to the various life threatening complications such as cardiovascular diseases, dyslipidemia, neuropathy, nephropathy, retinopathy, atherosclerosis, hypertension [16, 17] vascular inflammation and endothelial dysfunction [18, 19]. Obesity and sedentary lifestyle are other risk factors in the development of diabetes and cardiovascular diseases [20]. Pharmacotherapy is available for treatment of diabetes in the modern healthcare system which include insulin and oral hypoglycemic drugs [21]. However, due to economic constraints of various people, it is not possible to take these medications on the daily basis. Plants are considered a potential healer from ancient times probably because they promote the repair mechanism in the natural way [22]. Various herbs, rind of fruits, plants, stems, leaves and spices have been indicated for the diabetes management [23, 24 and 25]. Over 150 plants extract and some of their active principles including flavonoids, tannins, alkaloids, glycolipids and amino acids are used and very few of these plants have been screen pharmacologically [26, 27].

Streptozotocin induced diabetes model is an important and valuable model for induction of diabetes mellitus in experimental animals. Streptozotocin is believed to exert toxicity to beta cells in the pancreas. Basically, it probably does this by making pancreas swell and causing damage to beta cells in islets of Langerhans in the pancreas in 2-4 d after induction. Streptozotocin induces one type of diabetes which is similar to diabetes mellitus with non-ketosis hyperglycemia in some animal species [28]. However, diabetic animals survive without insulin treatment and shows improvement by oral hypoglycemic drug glibenclamide. This drug act by stimulating beta cells in the pancreas to release insulin and preventing their further destruction. In this model, diabetic rat shows symptoms like hyperglycemia, glycosuria, polyuria and loss of body weight which can be easily detectable in animals.

In the present study, STZ induction significantly elevated blood glucose level in rats. Chronic treatment with the methanolic extract of Citrullus lanatus (MECL) reduced elevated blood glucose level throughout the experimental period in the dose-dependent manner indicating its antihyperglycemic activity. In diabetes, body weight is probably reduced as body cells are unable to utilize glucose properly as a source of energy. So proteins are utilized as the energy source which in turn leads to decrease in protein content of the body and if protein content is reduced, there is reduction in body weight [29]. In the present study, body weight is instantly reduced in rats after induction of diabetes and remained underweight throughout the experimental period. Oral treatment with MECL throughout an experimental period, significantly elevated body weight in diabetic rats as compared to the diabetic control. This effect may be due to possible role of active principal of extract in the restoration of protein metabolism. Further in diabetes, due to persistent hyperglycemia, the excess blood glucose reacts with hemoglobin to form glycosylated hemoglobin. Since glycation rate is directly proportional to the blood glucose concentration, level of glycosylated hemoglobin indicates glycemic control in diabetic state [30]. In this study, oral treatment of MECL throughout the period significantly reduced the elevated glycosylated hemoglobin level in STZ diabetic rats. This effect further extends its potential in long-term glycemic control of diabetes mellitus.

In the study, decrease in glycogen content in the liver is due to disturbances in glycogen synthetase system. Improvement in liver glycogen of diabetic rats after chronic treatment with MECL indicates that it may be due to the improvement of glycogenesis or suppression of glycogenolysis. It has already been reported that 80% of death occur in diabetes is due to coronary artery disease associated with abnormal lipid or fat metabolism and altering lipid profile. Metabolic disturbances of lipid, carbohydrate and protein in diabetes cause lipolysis in adipose tissue leading to increase in serum cholesterol, triglyceride, LDL and VLDL [31]. The present study showed the increase in serum triglyceride, total cholesterol, LDL, VLDL in STZ-diabetic rats. Chronic treatment with MECL seeds restores all this parameter with an increase in HDL. So the watermelon seeds can be helpful in improving lipid metabolism in diabetes and will be effective to prevent diabetes-induced cardiovascular complications.

CONCLUSION

The present study showed that the methanolic extract of Citrullus lanatus seeds significantly lowered elevated fasting blood glucose level in STZ induced diabetic rats without showing hypoglycemic effects in normal rats. The exact mechanism of Citrullus lanatus in treatment of diabetes remains unclear, but possibly methanolic extract may reduced blood glucose by either stimulation of insulin release from beta cells that are less affected due to impact of streptozotocin or may functioned normally even in the presence of streptozotocin. This antidiabetic effect can further be due to an increased utilization of glucose by tissues or by an improved sensitivity of target tissues for insulin or it may be due to improved metabolism of glucose. Further, Citrullus lanatus significantly reduced lipid profiles in diabetic rats, so it can be considered effective drug for preventing related cardiovascular or other complications on long-term basis.

Thus, the present study concludes that the Citrullus lanatus possesses remarkable antidiabetic and antihyperlipidemic effects in STZ induced diabetic rats and also prevention of associated diabetic complications. However, comprehensive research is required to find out an exact mechanism behind this effect and identify the active constituents in drug responsible for this effect.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest in publication of this paper.

REFERENCES